THE UNIVERSITY
of EDINBURGH

Burden and Epidemiological Characterisations of *Streptococcus suis* in Chiang Mai, Thailand

Sanigan Thongsawad

Submitted in fulfilment of the requirement of the degree of Doctor of Philosophy

The University of Edinburgh

2016

PhD – The University of Edinburgh 2016

Declaration

I declare that the research described within this thesis is my own work and that this thesis is my own composition

Sanigan Thongsawad

Edinburgh 2016

PhD – The University of Edinburgh – 2016

Abstract

The burden of *Streptococcus suis* infection in humans is increasing worldwide. In Thailand, *S. suis* is the second most commonly recorded zoonosis. The principal sources of human *S. suis* infection are pig and pork products. A detailed understanding of the epidemiological characteristics of *S. suis* and the burden of the disease may help improve prevention and control policy to reduce the burden of this bacterial infection.

The work presented in this thesis focuses on human outbreaks of *S. suis* in Chiang Mai, Thailand, in humans and backyard pigs. This thesis examined the characteristics of previous outbreaks of *S. suis* in humans and calculated the incidence, disease burden and the associated economic burden of *S. suis* infection in Chiang Mai. The backyard pig system is important for *S. suis* transmission and this thesis examined the characteristics of the backyard pig production system in Chaing Mai and examined the prevalence and risk factor for *S. suis* infection in pigs. Finally, to examine transmission of *S. suis*, isolates collected during this study were identified and subject to molecular characterization.

A retrospective analysis of surveillance data for *S. suis* cases in Chiang Mai between 2005 to 2014 highlighted the annual incidence rate over this ten year period of 15.52 per 1,000,000 population, 6.5 times higher than for the rest of Thailand (2.37 per 1,000,000 population). The case fatality rate was high at 10.12%. The impact on human health of *S. suis* infection was derived from surveillance data for the year 2013. The health burden measured in term of Disability Adjusted Life Years (DALYs) was estimated at 7.41 per 100,000 population. Most of the health burden (98.28%) was in adults aged 15-64 years. Males had 3.5 times the health burden of females. The consequences of hearing loss and deafness had significant impacts on affected individuals quality of life. The economic impact of *S. suis* outbreaks in Chiang Mai was between 2013 and 2014 was estimated from interview data. Most patients

were covered for their health costs by the national health security scheme, with expenditure due to *S. suis* on average being 37,955 baht (£759) per patient. Out of pocket expenses for individuals and their families averaged 5,198 baht (£104) per patient.

An epidemiological survey of backyard pig production facilities was undertaken in Chiang Mai province where there was a reported high incidence of *S. suis* cases in humans occurred each year. Most holdings had between one to five pigs and all holdings shared similar characteristics and management practices. The prevalence of *S. suis* was in pigs was 4.8% (95%CI=2.2-7.4%). Pigs living in larger spaces (≥ 1.2 m2) showed a lower risk for *S. suis* infection (OR = 4.35, 95% CI = 1.07-25.21).

Examination of the isolates from this study revealed a diversity of serotypes. Only one isolate was identified as *S. suis* serotype 9. The rest did not match any common serotypes for *S. suis* (1, 2, 7 or 9) and known virulent strains were not identified. Twelve independent sequence profiles were determined by MLST, of which, 11 were novel. Backyard pigs were found to be commonly infected with a range of previously unidentified *S. suis* and may be a significant reservoir of human infection.

Contents

List of Figures

List of Tables

List of abbreviations

ALE – Active life expectancy

CSF – Classical swine fever

DALE – Disability-adjusted life expectancy

DALY – Disability-adjusted life years

DFLE – Disability free life expectancy

DLD – Department of livestock development

DNA – Deoxyribonucleic acid

ELISA – Enzyme link immunosorbent assay

EMIT – Emergency medical institute of Thailand

GBD – Global burden diseases

GDP – Gross domestic product

HALE – Healthy-adjusted life expectancy

HIV/AIDS – Human immunodeficiency virus infection and acquired immune deficiency syndrome

HRST – Healthy system research institute

HYLL – Healthy years of life lost

IHR – International health regulation

IMS – Immunomagnetic seperation

LAMP – Loop-mediated isothermal amplification

MLST – Multilocus sequence typing

NHCO – National health commission office

NHSO – National health security office

NSO – National statistical office

OIE – World Organisation for Animal Health

PCR – Polymerase chain reaction

PFGE – Pulsed field gel electrophoresis

PHC – Primary health care

PMC – Primary medical care

PRRS – Porcine respiratory and reproductive syndrome

PRRSV – Porcine respiratory and reproductive syndrome virus

PYLL – Potential years of life lost

QALE – Quality-adjusted life expectancy

QALY – Quality-adjusted life years

SIRS - Stenic inflammatory response syndrome

SIV – Swine influenza virus

SMC - Secondary medical care

SRRT - Surveillance rapid and response team

ST - Sequence type

TMC - Tertiary medical care

WHO - World Health Organisation

YLD - Year live with disability

YLL - Year of life loss

Acknowledgements

I would like to express my sincere gratitude to my supervisors Prof. Sue Welburn, Prof. Michael Thrusfield and Dr. Ewan MacLeod for their continuous support my PhD studies, for their patience, motivation, and immense knowledge. Their guidance helped me throughout my research and writing of this thesis. I could not have imagined having better supervisors and mentors for my PhD study.

I would like to thank my committee chair, Dr. Kim Picozzi, for her professional insight, comments and encouragement and Dr. Louise Hamill for her advice on laboratory work. I would also like to thank Pauline McManus for all practical support throughout my PhD and I thank the postgraduate office who are always supportive when I needed assistance.

I am grateful to the Thai government scholarship scheme and the Department of Livestock Development, Thailand for making it possible for me to at the University of Edinburgh, the Office of Educational Affairs, the Thai Embassy Office in the UK, who took care of financial and other issues without delay. Special thanks to Dr. Thammawan Noonthaisong for encouraging me to apply for the scholarship and Dr. Wandee Kongkaew for her advice on applying to the University of Edinburgh.

Several people helped with: providing the data, collection of samples and interview, the laboratory testing, and preparation of maps and I thank them all for their contributions. Special mention goes to the support staff of the Veterinary research and development center (upper northern region), Dr. Natnicha Teeyasuksaet, Mr. Sawai Tonkanya, Dr. Watanasak Chamlakorn, Ms. Prontip Prommeung, Ms. Kamonrat Karutharoth, Ms. Yuwathida Kumbuth, for help in collecting samples and laboratory analyses, Dr. Wichitra Anukul for helping me on approval and ethical issue of data collection, Dr. Oraphan Arjkampa for her advice on making maps. Thanks also to Ms. Sutherat Mahasingha for sharing surveillance data and arrange

interviews with the hospital staff and thanks to officers at the hospitals for their valuable time and information to help me complete my research. My heart-felt thanks to Livestock officers at the provincial and district levels for guiding me in the field work and help collecting samples. Thanks to my key informants, I acknowledge and appreciate their help and transparency in providing information that has helped me complete this thesis. I am also grateful to colleagues at the Veterinary Research and Development Center and my many friends, you should know that your support and encouragement was worth more than I can express.

Finally, I wish to express my sincere and heartfelt gratitude to my grandmother, my parents and sister for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

CHAPTER 1-Literature Review

1.1 General Introduction to *Streptococcus suis*

Most of the emerging diseases that have been reported worldwide are zoonotic diseases (Liu, Cao and Zhu, 2014). Since zoonoses can infect both animals and humans, their burden is shared by both medical and veterinary communities. There are several *Streptococcus* species that have been proven to be zoonotic, including *Streptococcus equi* sub-species zooepidemicus, *Streptococcus iniae, Streptococcus canis* and *Streptococcus suis*. *S. suis* is a pig pathogen responsible for important economic losses to the pig industry (Goyette-Desjardins *et al.*, 2014). *S. suis* is also an emerging zoonotic agent in humans (Wertheim *et al.*, 2009a) but has largely been neglected when considering *Streptococcus* infections in humans (Segura, 2009). This perception has changed recently due to an increased acknowledgement of increased impacts of *S. suis* on human health. Reports of *S. suis* outbreaks across Asia, in China (Yu *et al.*, 2006), Thailand (Khadthasrima *et al.*, 2007a; Takamatsu *et al.*, 2008; Vilaichone, 2002) and Vietnam (Ho *et al.*, 2011; Mai *et al.*, 2008) have increased recognition of *S. suis* as an important public health threat. *S. suis* was the first and third leading cause of human bacterial meningitis in Vietnam and Hong Kong, respectively (Hui *et al.*, 2005; Mai *et al.*, 2008).

S suis can cause various clinical manifestations in pigs and humans. Aside from meningitis, infection with *S. suis* bacteria also causes arthritis, pneumonia, septicemia, endocarditis and septic shock. (Feng *et al.*, 2007; Fongcom *et al.*, 2009; Kerdsin *et al.*, 2011a; Ma *et al.*, 2008; Wertheim *et al.*, 2009a). *S. suis* is a zoonosis that has worldwide distribution and infections in humans have been reported in all continents with the expection of Africa. Human infections have reported from been be found worldwide and infections in humans has been reported from China (Yu *et*

al., 2006), Hong Kong (Ma *et al.*, 2008), Japan (Chang *et al.*, 2006), Korea (Oh and Song, 2012), Singapore (Goyette-Desjardins *et al.*, 2014), Thailand (Takamatsu *et al.*, 2008) Vietnam (Mai *et al.*, 2008)), Croatia (Kopić, Paradzik and Pandak, 2002), Denmark (Perch, Kristjansen and Skadhauge, 1968), Germany (Heidt *et al.*, 2005), Italy (Princivalli *et al.*, 2009), Netherlands (van Samkar *et al.*, 2015), United Kingdom (McLendon, Bron and Mitchell, 1978)), Canada (Haleis *et al.*, 2009), United States (Fowler *et al.*, 2013)), Australia (Tramontana *et al.*, 2008), New Zealand (Dickie *et al.*, 1987)), Argentina (Callejo *et al.*, 2014) and Chile (Koch *et al.*, 2013)).

S. suis, is known as the "deafness disease" in Thai. Infection is associated with mortality and severe consequences, even though the morbidity is low. *S. suis* is associated with pigs, and outbreaks usually occur in the northern part of Thailand where some groups of people have the habit of consuming raw or semi-cooked meat and blood (Takeuchi *et al.*, 2012). Traditional raw pork dishes that are popular in this region are "Laab" (raw pork meat with spices), "Loo" (raw pork meat with blood and spices) and "Nham" (fermented raw pork). Outbreak investigations by the Surveillance Rapid Response Team (SRRT), Ministry of Health showed that consumption of raw or semi-cooked meat and blood was the most important risk factor for *S. suis* infection. High mortality rates of more than 70%, were found in patients that developed toxic shock syndrome and those who survived usually experienced a sequelae of hearing loss and vestibular disorder (Huong *et al.*, 2014; Lun *et al.*, 2007).

S. suis is one of the major public health zoonosis in Thailand. Public health campaigns and knowledge management are not effective in areas with sporadic cases and these interventions do nor reduce morbidity and mortality in humans. *S. suis* is not a major concern for animal health authorities, and there are no control measures

implemented on the animal side. The Thai Ministry of Health has reported around 150-200 *S. suis* cases each year since 2010, with mortality of 0.015 and is associated with high rates (54-80%) of temporary to permanent hearing loss. An understanding of the characteristics of *S. suis* disease outbreaks, as well as the population structure of *S. suis* will help improve *S. suis* surveillance and reduce *S. suis* human cases. Moreover, an understanding of the burden of this disease will help policy makers realise the important of prevention of *S. suis*.

1.2 Epidemiology of *Streptococcus suis*

As a major zoonoses, *S. suis* infection has been reported worldwide both in humans and animals. Pigs, which are a major reservoir of infection, become infected mainly through inhalation and the bacteria remain localised in the upper respiratory tract, particularly the palatine tonsils and nasal cavities. In addition, the genital and alimentary tracts are also natural habitats for *S. suis* (Gottschalk and Segura, 2000).

Porcine *S. Suis* is reported in countries where pigs are raised on a commercial scale; whereas human *S. suis* infection is reported mainly from Europe and Asia. The global cumulative incidence of *S. suis* infection in humans to 2012 showed that Thailand contributed the most of the cumulative incidence rate (8.21 cases/million population), followed by Vietnam (5.40 cases/million population) and Netherlands (2.52 cases/million population) (Huong *et al.*, 2014).

Aside from pigs and humans, *S. suis* has been isolated from a wide range of animal species including wild boar (*Sus scrofa*), cattle (*Bos Taurus*), horses (*Equus caballus*), dogs (*Canis lupus familiaris*), cats (*Felis catus*) and birds (*Aves*) (Devriese *et al.*, 1994; Devriese and Haesebrouck, 1992; Higgins *et al.*, 1990; Muckle *et al.*,

2010; Okwumabua *et al.*, 2017). There is a report of *S suis* in a dog whose infection may have come from ingestion of commercial pig ear treats (Muckle *et al.*, 2010).

The serotype of *S suis* also varies in each animal species as show in Table 1-1**Error! Reference source not found.**.

Table 1-1 *S. suis* serotypes found in animals

Animal	Serotypes
Human	1, 2, 4, 14, 16, 21
Pigs	1-34 and 1/2
Cats	4, 9, 20, 22, 26, 1/2
Birds	9
Ruminants	9, 10, 18, 20, 33
Wild boar	1-9, 15, 16, 20-23, 26-29, 31, 33, 34, 1/2

S suis has 33 known serotypes. Type 2 is considered the most pathogenic in both humans and pigs. Serotypes 1 - 9, 14 and 1/2 are responsible for infection in pigs, while serotypes 1, 2, 4, 14 and 16 cause disease in humans (Haleis *et al.*, 2009; Nghia *et al.*, 2008; Reams *et al.*, 1996; Wertheim *et al.*, 2009a). *S. suis* serotypes have not been identified for *S. suis* in the dog (*Canis lupus familiaris*), raccoon dog (*Nyctereutes procynoides*) or deer (*Cervidae*).

There is geographical variation in the distribution of *S. suis* serotypes (see Table 1-2). *S. suis* serotype 2 is found worldwide and it is the most prevalent. Other serotypes are found in specific regions; serotype 14 is mainly found in Europe and North America, while serotype 16 is found in Southeast Asia (Fittipaldi *et al.*, 2011; Goyette-Desjardins *et al.*, 2014).

Table 1-2 Geographic distribution of *Streptococcus suis* serotypes

Area	Serotypes in animals	Serotype in humans
Worldwide	2	2
Asia	1/2, 1-4, 8-16, 20, 22, 23, 25, 26, 28, 29, 33	2
Southeast Asia	½, 1-5, 7-9, 14, 16, 17, 21, 22, 31	2, 16
Europe	½, 1-9, 12, 14-16, 22, 23, 26-29, 31, 33, 34	2, 5, 14
North America	½, 1-8, 10	2, 14
South America	½, 1, 2, 7-9, 11	2
Australia	½, 1-4, 7, 9	2

Human *S. suis* infections are associated with either pig contact or consumption of contaminated pork via wounds or through mucosa. The majority of cases in Northern Europe and Southeast Asia are reported in areas of intensive pig rearing for the (Wertheim *et al.*, 2009a). Infection can be caused by direct contact with infected carrier pigs, sick pigs, or consumption of raw pork contaminated with *S. suis*. Human infection from occupational exposure, affects pig farmers, abattoir workers, meat inspectors, meat processing workers, butchers and veterinarians (Fowler *et al.*, 2013; Robertson and Blackmore, 1989; Smith *et al.*, 2008; Yu *et al.*, 2006). Risk estimates for *S. suis* infection in humans have been made in the Netherlands where abattoir workers had the highest risk of 3.8 per 100,000 people, follow by pig breeder (2.7) and butchers (1.2) (Arends and Zanen, 1988).

S. suis infections related to pork and blood consumption commonly occur in countries that have high levels of pork consumption; including Hong Kong, Thailand and Vietnam (Teekakirikul and Wiwanitkit, 2003; Mai *et al.*, 2008; Wertheim *et al.*, 2009a; Takeuchi *et al.*, 2012). A study in Hong Kong found that individuals with

direct contact to pigs or that ingested raw pork had the highest risk of infection, at 32 per 100,000 people (Ma *et al.*, 2008).

1.3 Recognition of *Streptococcus suis*

S. suis was first reported after an outbreak of arthritis and meningitis in piglets in the Netherlands in 1951 (Jansen and Van Dorssen, 1951). Seventeen years later, the first human case was reported in 1968 in Denmark (Perch, Kristjansen and Skadhauge, 1968). The first two large outbreaks of *S. suis* were reported from the Netherlands in 1988 and Hong Kong in 1995, with 30 and 21 cases respectively. Most cases had history of pig contact and the patients presented with meningitis with 50% suffering hearing loss, with a mortality rate of less than 7% (Kay, Cheng and Tse, 1995; Arends and Zanen, 1988). Sporadic human cases of *S. suis* are now consistently reported worldwide.

East and Southeast Asia are considered endemic for *S. suis* (Goyette-Desjardins *et al.*, 2014). Most *S. suis* cases (90%) occur in Asia with Vietnam, Thailand and China accounting for 83.6% of all global cases. Between 1996-2005, southern Vietnam reported 151 patients with *S. suis* infection (one case of serotype 14 and the remainder serotype 2) of which 33.1% of cases were exposed to pigs or pork products. The infection was an important cause of morbidity, attributed to hearing loss (Mai *et al.*, 2008).

The largest outbreak of 215 cases of *S. Suis* (66 laboratory-confirmed) including 38 deaths, was reported in Sichuan Province, China, between July and August 2005. Those affected were backyard farmers who had been directly exposed to infection while slaughtering pigs that had either died of unknown causes or were

sick and were killed for consumption (Yu *et al.*, 2006). The *S. suis* identified in this outbreak were genetically related to an outbreak of 25 cases in Jiangsu province in 1998, the first *S. suis* outbreak in China that resulted in 14 deaths, and a meningitis case in identified in Hong Kong in 1996 (Ye *et al.*, 2006, 2008). The causative agent was identified as a clone of *S. suis* serotype 2, sequence type (ST) 7 (Ye *et al.*, 2006). These outbreaks were closely related to a large regional outbreak of *S. suis* infection in pigs, all human cases in Jiangsu and Sichuan Provinces occurred in areas endemic for *S. suis* in pigs, and approximately 80,000 pigs were estimated to be have been infected by *S. suis* at this time (Lun *et al.*, 2007).

1.4 The causative agent

Streptococcus suis are a group of encapsulated gram-positive, facultative anaerobes that usually occur as pairs or chains. Originally, *Streptococcus* which was known to causes meningitis, pneumonia, arthritis, endocarditis, septicemia and abortion in pigs, were classified by its cell wall into Lancefield groups R, S, RS and T. Since 1987 *Streptococcus suis* has been recognized as a species and classified in Lancefield group D (Klipper-Balz and Schleifer, 1987). *S. suis* are classified by serotype; the former Lancefield group R, S, RS and T corresponding to serotypes 2, 1, ½ and 15, respectively.

There are 33 known serotypes of *S. suis* based on their polysaccharide capsular antigen, serotypes 1-31, 33 and ½. Serotypes 32 and 34 have been reclassified as *Streptococcus orisratti* (Perch, Pedersen and Henrichsen, 1983; Gottschalk *et al.*, 1989; Gottschalk, Higgins and Beaudoin, 1990; Gottschalk *et al.*, 1991; Higgins *et al.*, 1995; Hill *et al.*, 2005). A recent study examining the genes encoding manganese-dependent superoxide dismutase (*sodA*) and the recombibation/repair protein (*recN*)

indicated that the reference strains serotypes 20, 22, 26 and 33 should be taxonomically removed from *S. suis* (Tien *et al.*, 2013). Serotype 20, 22 and 26 have been proposed as a novel species, *S. parasuis* (Nomoto *et al.*, 2015). Among all *S. suis* capsular types, serotype 2 is the most prevalent in diseased pigs in most countries and it the serotype most frequently associated with disease in humans.

S. suis is susceptible to β-lactam antibiotics including penicillin, ampicillin, amoxicillin, cephalosporin and ceftriaxone (Markowska-Daniel *et al.*, 2010), but has evolved resistance to tetracycline (>90%) and macrolides (>70%) (Princivalli *et al.*, 2009; Hoa *et al.*, 2011; Palmieri *et al*., 2011; Chen *et al.*, 2012; Callens *et al.*, 2013).

S. suis can withstand environmental stress. Bacteria can survive for more than 10 days at room temperature in porcine tissue or body fluids (whole blood, brain tissue, urine and semen) and for up to six weeks in carcasses at 10^{o}C (Clifton-Hadley *et al*., 1986; Dee and Corey, 1993). *S. suis* can survive within in faeces for up to 104 days at 0^{o}C and for up to eight days at room temperature. In dust the bacteria can survive at 0^{o}C for 54 days, but *S. suis* cannot be isolated from dust at room temperature after 24 hours (Clifton-Hadley and Enright, 1984).

S. suis is easily destroyed by disinfectants such as phenol, quaternary ammonium, formaldehyde, chlorhexidine, 3% iodine and 5% hypochlorite but is resistant to 70% alcohol (Dee and Corey, 1993).

1.5 *S. suis*, a commensal bacterium in pigs

The pig microbiota, the ecological community of commensal, symbiotic and pathogenic microorganisms (Lederberg and McCray, 2001) comprises all microorganisms (bacteria, viruses and fungi) that reside in the body e.g. respiratory

tract, gastrointestinal tract, and other mucosal surfaces. The aggregate of resident microorganisms is referred to as the commensal microbiome. Microbiota play a key role in influencing immune reactivity (Littman and Pamer, 2011; D'Argenio and Salvatore, 2015). Microbiota help protect from infection, allowing the immune system to recognise bacteria harmful to the host and permitting other, helpful bacteria to carry out their functions, e.g. nutrient acquisition, immune maturation, and neurological function (Rhee, Pothoulakis and Mayer, 2009; Round and Mazmanian, 2009; Zhang and He, 2015). Under normal conditions, the resident microbiota cause the host no harm. Some microbes may become opportunistic pathogens and cause disease.

The surface tissue of a healthy animal, e.g. the skin and mucous membranes are colonized by a variety of microbial species, but bacteria are the most numerous, comprising a diverse reservoir community of commensals and potential pathogens. Some may be pathogenic e.g. *S. suis*, *Staphylococcus aureus*, and *Enterobacteriaceae* (Goyette-Desjardins *et al.*, 2014; Szmolka and Nagy, 2013; Smith *et al.*, 2013), with varied potential for virulence, depending on the strain and the immune status of the host (Littman and Pamer, 2011). *S. suis* are one of the most important bacterial species in pigs, representing both normal flora and opportunistic pathogens. Almost every pig is colonised by commensal strains of *S. suis* in the gastrointestinal and reproductive tracts (Wertheim *et al.*, 2009a). However, a more virulent strain of the bacteria also exists and causes disease in pigs.

Many studies have attempted to understand how commensal bacteria become pathogenic. In some cases, there may be an environmental trigger or temporal cue that stimulates bacterial activity, resulting in infection or disease (Littman and Pamer, 2011). Commensal bacteria may become pathogenic following a secondary infection

(Bosch *et al.*, 2013), e.g. a human infected with an influenza virus releases *S. pneumoniae* bacterium from the biofilm, resulting in respiratory disease (Chao *et al.*, 2015). Primary viral infections can result in *S. suis* pathogenicity and increase disease severity, e.g. co-infection with Porcine respiratory and reproductive syndrome (PRRS) and swine influenza (Meng *et al.*, 2015; Wang *et al.*, 2013; Dang *et al.*, 2014; Thanawongnuwech *et al.*, 2000). Swine influenza can promote colonization and invasion of *S. suis* (Meng *et al.*, 2015) and in many PRRS outbreaks, there has been an increase in susceptibility of pigs to secondary bacterial infections, including those of *S. suis* (Wu *et al.*, 2015; Huong *et al.*, 2016). Examination of the interaction between influenza and *S. suis* revealed that infection of porcine tracheal cells by SIV facilitated adherence of *S. suis*. A haemagglutinin of SIV recognized α2,6-linked sialic acid present in the capsular polysaccharide of *S. suis*. (Wang *et al.*, 2013; Wu *et al.*, 2015).

Porcine respiratory and reproductive syndrome virus (PRRSV) infection also increase susceptibility to *S. suis* infection, negatively affecting the immune system. Co-infection with PRRSV and *S. suis* significantly increased the pathogenicity of *S. suis* (Xu *et al.*, 2010; Galina *et al.*, 1994). Leukocytes, lymphocytes, and monocytes were significantly depleted in pigs infected with PRRSV and severe cortical depletion of thymocytes was also observed (Feng *et al.*, 2001).

Co-infections with *Haemophilus parasuis* and *Aerococcus viridans* (commensals in healthy pigs) can also result in *S. suis* becoming pathogenic (Barre, 2015; Pan *et al.*, 2016; Martín *et al.*, 2007). Barre (2015) found that *S. suis* growth was enhanced in the presence of *H. Parasuis* (*H. parasuis* being disadvantaged). *H. parasuis* and *S. suis* biofilms were decreased by co-culture. Virulence may be

increased by preventing entrance into a quiescent biofilm form, and by offering a synergistic protection against antimicrobials and complement.

A. viridans and *S. suis* were recently isolated from the brains of piglets that had suffered from bacterial meningitis (Pan *et al.*, 2016). Co-infection of *S. suis* with *A. viridans* in mice enhanced the ability of a new serotype of *S. suis* that caused bacterial meningitis and death (Pan *et al.*, 2016).

1.6 Pathogenesis and clinical manifestation

The pathogenesis of *S. suis* is not well understood. Most studies have been limited to *S. suis* serotype 2 and development of bacterial meningitis. Entry sites for *S. suis* may be open cuts and abrasions and via the epithelial cells of the respiratory tract and intestine. The mechanisms by which *S. suis* traverse the mucosa and blood-brain barrier and travel in the bloodstream are unknown (Gottschalk and Segura, 2000). Studies suggest that bacterial-viral interactions, such as H1N1 swine influenza virus, markedly increase the ability of *S. suis* to adhere, invade and activate respiratory epithelial cells (Dang *et al.*, 2014; Wang *et al.*, 2013). Pathogenic bacteria are exposed to temperature changes during colonization and RNA thermometers are reported to be associated with regulation of virulence-associated proteins but there are no means to measure the effects of RNA structures in gene regulation during bacterial infection (Grosso-Becera, Servín-González and Soberón-Chávez, 2015).

The gastrointestinal tract is a major route for infection by *S. suis* in humans, particularly in some Asian countries where ingestion of raw or uncooked pork is a risk factor. Intestinal bacterial translocation may offer a pathway for *S. suis* infection. Adherence of *S. suis* to intestinal epithelial cells is associated with both serotype and

genotype (Ferrando and Schultsz, 2016; Swildens *et al.*, 2004). Liver disease and/or alcohol consumption are risk factors for translocation of *S. suis* from the intestine (Nakayama *et al.*, 2013).

Environmental conditions potentiate disease spread (Patz *et al.*, 2003) and the importance of meteorological data in modelling the occurrence of porcine pasteurellosis has been recently been demonstrated (Gao *et al.*, 2016). *S. suis* together with predisposing infections e.g. PRRS may also follow an airborne route.

More than seventy virulence factors are associated with *S. suis* pathogenesis including; suilysin, arginine deiminase system, glutamate dehydrogenase, and permease (Fittipaldi *et al.*, 2012).

The most common clinical signs for *S. suis* infection in humans and pigs are meningitis and septicaemia. These and other clinical signs vary among other animal species and are summarised in Table 1-3.

Table 1-3 Animals infected with *Streptococcus suis* and their clinical signs.

Animal	Clinical signs
Human	Meningitis, deafness, septicemia, epicarditis, toxic-shock syndrome
Pigs	Meningitis, arthritis, septicemia, pneumonia, endocarditis
Cats	Pneumonia, moist dermatitis, meningoencephalitis
Birds	Septicemia with multiple sudden deaths
Horses	Meningitis, guttural pouch, pneumonia, osteomyelitis
Ruminants	Meningitis, arthritis, pneumonia, peritonitis, septicemia
Deer	Peritonitis, septicemia

1.6.1 Clinical manifestations of human infections

S. suis infections can be asymptomatic or acute with an incubation period varying from a few hours to several days. Infection in humans have been associated

with both purulent and non-purulent meningitis. Common clinical manifestations includes septicaemia, toxic shock syndrome, arthritis, endocarditis and spondylitis (Feng *et al.*, 2007; Fongcom *et al.*, 2009; Kerdsin *et al.*, 2011a; Ma *et al.*, 2008; Wertheim *et al.*, 2009a). Early symptoms resemble influenza and cases may experience arthritis in multiple joints, several days before any other signs manifest. These symptoms are followed by clinical symptoms of meningitis such as headache, high fever, neck stiffness, nausea, vomiting, vertigo, photophobia, ataxia and articular pain. Endocarditis, cellulitis, rhabdomyolysis, arthritis, pneumonia, otitis, skin hemorrhage, toxic shock syndrome and endophalmitis have also been reported.

The incidence of deafness after *S. suis* infection is higher than that for any other meningitis causing bacteria. Early eight cranial nerve damage is commonly observed in patients with *S. suis* meningitis, manifesting as hearing loss and vestibular dysfunction (Shneerson *et al.*, 1980; Rusmeechan and Sribusara, 2008). Hearing loss is usually observed within 24 hours of infection and deafness following meningitis and vestibular dysfunction occurs in 50% of survivors (Donsakul *et al.*, 2003; Dejthevaporn and Witoonpanich, 2003; Navacharoen *et al.*, 2009). A recent review of *S. suis* cases in the Netherlands indicates hearing loss may be far more common, occurring in 86% of cases (van Samkar *et al.*, 2015).

Death occurs with severe cases of septicemia with systemic inflammatory response syndrome (SIRS) including multiple organ failure, toxic shock syndrome, disseminated intravascular coagulation and associated purpura fulminans. Mortality rates for *S. suis* are variable and can reach 18% (Smith *et al.*, 2008).

1.6.2 Clinical manifestations in the pig

S. suis is an opportunistic bacteria that is part of the normal flora in the nasal cavity, tonsils, genital and alimentary tract of healthy pigs (Gottschalk and Segura, 2000). The bacteria usually colonisine the upper respiratory tract of adult pigs without causing any disease (Padungtod *et al.*, 2010).

S. suis can cause severe bacteremia in piglets and is responsible for large economical losses in the swine industry (Byra *et al.*, 2011). High bacteremia in the circulatory system causes meningitis or septic shock with multiple organ failure (Straw, 2006). Stress caused by poor management, over-crowding and inadequate ventilation cause multiplication of bacteria and the spread from the tonsils to the lymphoid system (Neumann *et al*., 2009). Although *S. suis* can infect pigs of all ages, infections occur more frequently in early weaned pigs at 5-6 weeks of age (Amass *et al.*, 1996), when maternal immunity drops below protective levels and when pigs are susceptible to other immunosuppressive diseases such as PRRS, enzootic pneumonia or swine influenza (Neumann, Ramirez and Schwartz, 2009). Co-morbidity of *S. suis* with other immunosuppressive diseases is associated with more severe clinical symptoms (Galina *et al.*, 1994; Thanawongnuwech *et al.*, 2000).

Swine infected with *S. suis* show similar clinical symproms to those in humans including; septicaemia, encephalitis, meningitis, pneumonia and arthritis (Staats *et al.*, 1997). Disease severity depends on the virulence of the agent and pig immunity (Vecht *et al.*, 1992). Symptoms associated with septicaemia and meningitis may resulting in death in early weaning pigs. Clinical disease progression goes through depression, fluctuate fever, shifting lameness, and neurological signs including shivering,

incoordination, inability to stand, paddling, opisthosomas, convulsions, and nystagmus (Gottschalk, 2014). Blindness and deafness may occur (Neumann, Ramirez and Schwartz, 2009). Sudden death from acute septicemia in the absence clinical signs can occur (Gottschalk, 2014).

1.7 Disease transmission

In humans, the most significant route of transmission of *S. suis* from pigs to is through broken skin by contact with infected pigs or handling infected pork. The risk is higher in immune-compromised individuals including: HIV/AIDS patients; chemotherapy patients, splenectomy patients or transplant recipients on medication. Transmission also occurs via the gastrointestinal tract from consumption of raw pork and blood from infected pigs. There is no evidence of human to human transmission.

S. suis spreads from pig to pig by nose to nose contact and by indirect aerosol infection in pigs in confined spaces. *S. suis* can be introduced by healthy carrier pigs that harbor the organism in their tonsils or nasal passages for months. Sows can transmit organism to piglets via vaginal secretion during parturition and while nursing (Gottschalk, 2010). The main factors that lead to *S. suis* spread in farms are poor ventilation control and excessive crowding. Environmental contamination may play a role in transmission since the organism can survive long periods in feces, dust, carcasses, and flies (Dee and Corey, 1993).

In Hong Kong and China, *S. suis* is more commonly isolated during the summer (Ma *et al.*, 2008; Yu *et al.*, 2006). Transportation during hot and humid weather might cause additional stress to pigs, rendering them more susceptible to infection (Chau, Huang and Kay, 1983). Hot and humid temperatures might also

facilitate multiplication and overgrowth of *S. suis*. Any immunosuppressive diseases within the herd may cause more serious problems. Clinical cases can arise from the introduction of new strains and virulent strains may already be present in the herd, presenting no clinical signs. *S. suis* can easily be killed easily by common disinfectants used in farm including phenolic, chlorine and iodine based disinfectants.

1.8 Diagnosis of *Streptococcus suis*

1.8.1 Differential diagnosis in humans

S. suis infection is similar to infection with a range of other bacteria. Acute meningitis, caused by *S. suis* is clinically similar that observed with infection of *S. pneumoniae* or tubercular subacute meningitis. Sepsis is common to other gram-positive bacterial infections: *Streptococcus aureas* and β-hemolytic *Streptococci* infection. The clinical signs of primary peritonitis; arthritis; toxic shock and endocarditis also occur in patients with cirrhosis.

A presumptive diagnosis of *S. suis* is based on combining history, clinical signs, and laboratory confirmation. Patient history is important and can support diagnosis. Almost patients are male, middle-age with drinking habit or have careers related to pig production such as farmers, butchers, meat processing workers, or have history to contact or consumption of raw pork or blood.

The incubation period for *S. suis* is usually around 2 days but can vary from a few hours to 14 day (Dragojlović *et al.*, 2005; Wertheim *et al.*, 2009a; Takeuchi *et al.*, 2012). *S. suis* infected patients will commonly present with acute or subacute meningitis. Patients may be observed with clinical signs related to central nervous system damage including a decrease of hearing ability, vertigo, nystagmus, that

enables differentiation of *S. suis* infection from meningitis induced by *S. pneumoniae* infection or tuberculosis. A third of *S. suis* patients, may have cellulitis, fasciitis or arthritis. In some cases, patients may present with uveitis and patchy or ecchymosis. The confirmation of a *S. suis* infection is by isolation of the bacterial from patient.

1.8.2 Differential diagnosis in animals

In pigs, the symptoms of *S. suis* infection can also resemble many other infectious diseases, including Glasser's disease, caused by *Haemophilla parasuis*, and Edema disease caused by *Escherichia coli*. These diseases present with similar neurological signs and respiratory signs including coughing, sneezing, or abdominal breathing, especially when co-infections are present.

Post-mortem lesions can help inform a pathological diagnosis. *S. suis* infection is commonly associated with petechial hemorrhaging in all parts of the lung, multiple joint arthritis with white to yellow pus in the joint, endocarditis in some cases, leptomeninges pus covering the hind brain, serositis and fibrin covering internal organs. However, for definitive diagnosis laboratory tests are needed.

1.8.3 Laboratory diagnosis of *S. suis*

For a diagnosis of bacterial meningitis, cerebrospinal fluid (CSF) culture is the "gold standard". A definitive diagnosis of *S. suis*, requires isolation of the bacteria. For cases where there is no culture obtained from the CSF, gram staining, ELISA and PCR can be applied. Where CSF cannot be obtained or when patients have clinical signs besides meningitis, blood or specimens obtained from lesions may be suitable for analysis. Results are always interpreted with the case history. Since treatment cannot be delayed, a primary diagnosis of *S. suis* infection is normally based on clinical

signs, symptoms (meningitis, septicemia, endocarditis, deafness), case history and assessment of risk factors associated with pig contact or raw pork consumption.

The most suitable specimens for *S. suis* testing are CSF, blood and samples obtained from lesions (joint fluid, pleural fluid or other sterile site specimens). Most *S. suis* patients show signs of septicaemia or meningitis. In cases of acute sepsis or meningitis, blood samples are drawn from two sites and cultured independently. In the case of sub-acute endocarditis or chronic bacteremia, blood should be collected at two time points at least one hour apart. Blood should be collected before antibiotics are administered where possible or of not, blood should be taken when the antibiotic levels are lowest level in bloodstream.

In pigs, samples are usually collected from organs showing lesions. In the case of septicemia; samples from the spleen, liver and kidney samples are collected from the carcass. If animals exhibit signs of meningitis, a brain swab or brain impression smear will be taken.

1.8.3.1 Bacterial identification

Bacterial culture and biochemical tests

Bacterial culture is used to determine the type of organism, with the aim to isolate a pure culture of a single species of bacteria. General purpose media supports the growth of many bacteria while enriched selective media are supplemented by blood or special nutrients to encourage the growth of fastidious hetorotrophs. The media routinely used for bacterial culture of CSF in meningitis patients include 5% sheep blood agar, enriched chocolate agar, and an enrichment broth e.g. thioglycolate, Columbia, brucella supplement peptone, or eugenic (Gray and Fedorko, 1992). *S. suis*

grows on selective blood or serum-enriched media with mucoid colonies (Rosendal *et al.*, 1986). Colonies on blood agar are slightly gray, 2-3 mm, moist and semitransparent. All *S. suis* strains present α-hemolysis on sheep blood agar, but may turn β on prolong incubation (Huh *et al.*, 2011). In Thailand, *S. suis* is cultured from CSF and blood from meningitis patients using sheep blood agar, chocolate agar and MacConkey agar. The culture is incubated at 35-37 ^{o}C with 5-7% CO_2 (*S. suis* can grow in normal conditions but it favors CO_2).

S. suis is often misidentified as the colonies look similar to other *Streptococcus* species including; viridans streptococci, *Enterococcus faecalis, Aerococcus viridans* and *Streptococcus pneumoniae* (Facklam, 2002). In a study in Thailand, 70% of cases ascribed to viridans *Streptococcus* cases were, in fact, *S. suis* (Fongcom *et al.*, 2009).

Definitive identification of bacteria normally requires further biochemical or molecular testing. Biochemical tests can differentiate *S. suis* and from other Streptococcus species. Parameters used to identify *S. suis* are oxidase negativity, catalase negativity, Voges-Proskauer negativity, non-motile, hydrolysis of optichin positivity, trehalose positivity, negativity for growth in 6.5% NaCl, some strains positivity for growth in 40% blie. *S. suis* serotype 2 is negative for hippurate, pyrrolidonylarylamidase and mannose (Tarradas *et al.*, 1994).

Several rapid test kits are available for the identification of *S. suis* including API Rapid Strep System and Vitek II system (bioMerieux) although capsular serotyping is still needed for definitive accuracy.

Polymerase Chain Reaction (PCR)

PCR tests can improve diagnosis in humans (Nga *et al.*, 2011; Nguyen *et al.*, 2007) and *S. suis* should ideally be confirmed using molecular methods (Heidt *et al.*, 2005). A multiplex PCR was developed to rapidly identify the serotype of *S. suis* (Kerdsin *et al.*, 2012, 2014; Liu *et al.*, 2013), which is routinely performed in patients with suspected *S. suis* meningitis.

ELISA

ELISA tests can be used to detect and differentiate *S. suis* in pigs and are provided by many manufactures. The antigen used can differentiate *S. suis* from other diseases including porcine rabies virus, porcine parvovirus, porcine reproductive and respiratory syndrome virus foot and mouth disease virus FMDV, *Salmonella, Mycoplasma*. However, cross-reaction between serotypes is an issue (del Campo Sepúlveda *et al.*, 1996). High antibody levels were found in infected pigs compared to normal pigs, even though these were not sufficient to differentiate infected pigs from the normal group (del Campo Sepúlveda *et al.*, 1996). ELISA cannot detect *S. suis* in the early stages of infection as antibody levels increases slowly to four weeks after infection (Lapointe *et al.*, 2002) .

Other techniques

Several new techniques have been developed to improve diagnostics for *S. suis*. *In situ* hybridization and immune-histochemical methods have been developed for detection in tissue sections, mostly brain, endocardium and lung (Madsen *et al.*, 2002; Boye *et al.*, 2000). Techniques identify serotypes of *S. suis* using specific probes, however, cross-reactions between serotypes can occur (Boye *et al.*, 2000).

Immunomagnetic Separation (IMS) can detect and identify *S. suis* serotype 2 and ½ in tonsils, nasal cavities and the genital tract (Gottschalk *et al*., 1999).

A loop-mediated isothermal amplification (LAMP) reaction has been developed for rapid detection of *S. suis* (Aschalew *et al.*, 2014; Zhang *et al.*, 2013). LAMP uses four specially designed primers that recognize a total of six distinct sequences on the target DNA. Amplification is achieved in a single step under isothermal conditions without the need for advanced instrumentation (Notomi *et al.*, 2000). LAMP could identify all known *S. suis* serotypes and was suitable for detection of *S. suis* in pork meat (Arai *et al.*, 2015).

1.8.3.2 Serotyping

Serotypes or serovars are variations within *S. suis*, classified as recognizable, antigenic differences in the capsular polysaccharide. The capsular polysaccharide synthesis (*cps*) locus encodes protein/enzymes responsible for capsular production; variation in the capsule structures and are the basis of *S. suis* serotyping (Liu *et al.*, 2013). Serotypes play an essential role in determining *S. suis* species. Two techniques (PCR and agglutination tests) are commonly applied to identify *S. suis* serotypes.

PCR

PCR tests can identify the species as well as serotypes of *S. suis*. PCR is based on detection of a specific conserved gene sequence, a 688 bp fragment of a housekeeping gene encoding *gdh* and a 294 bp sequence for the 16s ribosomal RNA (rRNA) sequence that can be used to identify *S. suis* strains (Marois *et al.*, 2004; Okura *et al.*, 2014; Okwumabua *et al*., 2003). Thirty three serotypes have been identified

using multiplex PCR (Kerdsin *et al.*, 2014; Liu *et al.*, 2013). A real-time PCR has been developed to detect *S. suis* serotype 2 in patients (Nga *et al.*, 2011).

Latex Agglutination test

The latex agglutination test has been used to identify and type bacterial meningitis. It is easy to perform and gives quick result. Tests are based on utilizing the serum which contains antibodies towards bacteria. The commercially available tests are designed to specific against the capsular polysaccharides of the bacteria. The latex agglutination test is the prominent serological technique for serotyping of *S. suis*.

1.9 *Streptococcus suis* control measures

1.9.1 Human behavior influencing the occurrence of *S. suis*

S. suis infection is an occupational disease in industrialised countries but is more commonly found to be a foodborne infection in Asia. Meta-analysis showed *S. suis* with occupational infection was 38.1%. This proportion was higher in economically developed countries. Occupational exposure in United Kingdom, the Netherlands and Japan accounted for 83.8% (Huong *et al.*, 2014). In the Netherlands, the annual risk of *S. suis* meningitis among abattoir workers and pig breeders is 1,500 times higher than within the general population (Arends and Zanen, 1988). Occupational exposure is also the main cause of *S. suis* infection in China and Hong Kong, outbreaks start during the slaughtering process of pigs or from handling of raw pork (Ma *et al.*, 2008; Yu *et al.*, 2006). Occupations that involve the handling of pigs including pig farmers, bleeders, abattoir workers, carcass cutting and processing workers, butchers and cooks are similarly at risk through cuts or abrasions on skin. Handling sick or dead pigs a major risk factor for *S. suis* (Yu *et al.*, 2005).

In Southeast Asia, mainly in Thailand and Vietnam, the proportion of patients with occupational exposure is lower than in Europe, but the risk is higher from consumption of meals containing raw pork with the pooled estimate of 37.3%. The proportion in Thailand alone was 55.8% (Huong *et al.*, 2014). Food consumption plays a major role in *S. suis* infection in this region, studies in Thailand and Vietnam confirmed that eating undercooked pork products was the most important risk factor (Ho *et al.*, 2011; Takeuchi *et al.*, 2012).

Predisposing factors for *S. suis* infection include alcoholism and diabetes mellitus (Rusmeechan and Sribusara, 2008; Ho *et al.*, 2011a). *S. suis* is likely to translocate across the intestines and brain barrier of humans who have liver disease and/or consume alcohol. Studies in mouse models have showed that the percentage of *S. suis* detected in blood of acute alcoholic and cirrhotic mice were two and four times higher than control mice respectively. Bacterial translocation was also observed in the brain (31.8%) and in alcoholism and cirrhosis group (62.5%), while no bacteria were detected in the healthy model mice (Nakayama *et al.*, 2013).

Previous medical illnesses that predispose immunosuppression are risk factors in the development of serious *S. suis* disease (Gottschalk, 2014) but are not essential for *S. suis* infection as the majority infected cases did not have any previous medical problems (Chang *et al.*, 2006; Rusmeechan and Sribusara, 2008).

1.9.2 Surveillance and investigation

S. suis is not a notifiable disease in most countries, however, is a notifiable disease in Thailand which has to report individual suspect cases to Ministry of Public Health (Chuxnum, 2009). In Hong Kong, *S. suis* was previously classified as an

occupational disease and needed to be reported to the Labor Department. However, after a large outbreak of *S. suis* in Sichuan in August 2005, medical practitioners in Hong Kong are now required to report suspected *S. suis* cases to the Center for Health Protection, Department of Health. (Hong Kong Special Administration Region Government, 2005). Similar notification is required in United Kingdom, the clinical *S. suis* syndrome is reportable under Reporting of injuries, Dangerous Occurrence Regulation (RIDDO) to Health and Safety Executive (HSE) (Department of Environment, Food and Rural Affairs, 2013).

1.9.2.1 Surveillance and control of *S. suis* in Thailand

S. suis is an important zoonotic disease especially in the upper northern part of Thailand, where large outbreaks have occurred and where people commonly eat traditional raw pork dishes. Public health officials have developed special surveillance systems to survey and control *S. suis* in this area that have been expanded to the whole country. The flow chart for *S. suis* surveillance is shown in Figure 1-1.

1.9.3 Prevention and Control

S. suis infection is a risk to those involved in meat production, to those who have contact with live pigs or handle uncooked pork (Robertson and Blackmore, 1989; Yu *et al.*, 2005). People who consume raw or semi-cooked pork are also at risk of infection (Ho et al., 2011; Neungmek and Pathanasophon, 2011). Prevention requires an integrated approach to food production from “farm to fork”, involving decreased infection in animals; avoid contamination along the food chain; as well as prevention in people working on food production and consumer (Papatsiros, 2011).

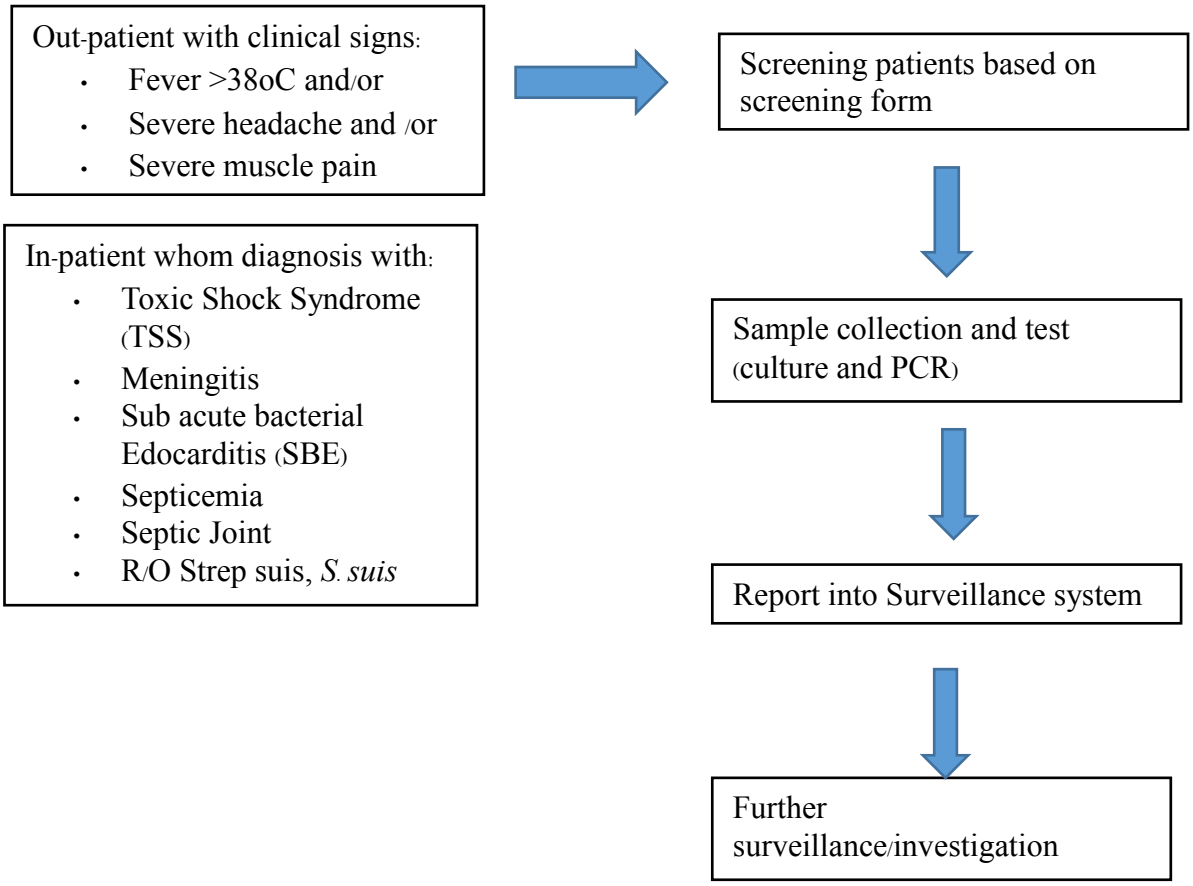


Figure 1-1 Flow chart for *S. suis* surveillance system in Thailand. (Adapted from: Guidelines to Control and Prevention of Emerging diseases for Public Health Personnel, 2011).

There is no vaccine for *S. suis* in humans and preventive measures are tailored to preventing transmission (Papatsiros, 2011). Education targeted at stakeholders in the pig production chain, raise awareness and help to reduce risk of *S. suis* infection in humans. Individuals handling pigs are recommended to use standard precautions to protect themselves. There is no evidence of human-to human transmission.

S. suis is the predominant cause of streptococcal disease in swine (Neumann, Ramirez and Schwartz, 2009), widespread in pig populations of pig industrial countries (Gottschalk *et al.*, 2010). The disease is usually seen in nursing or recently weaned piglets; although the bacteria can be found in pigs without clinical signs. *S.*

suis incidence on farms depends on management and farm size. Risk factors for *S. suis* in pigs include stress, usually from overcrowded, poor air ventilation, fluctuating temperatures, high humidity, poor hygiene and poor nursery management. Co-infection with other bacterial and virus diseases such as *Bordetella bronchiseptica*, *Pasteurella multocida*, pseudorabies, swine influenza, PRRS may potentiate disease outbreaks (Galina *et al.*, 1994; Thanawongnuwech *et al.*, 2000; Vecht *et al.*, 1989).

S. suis transmission can be by ingestion, inhalation or nose-to-nose contact. There is a low probability that fomites and flies may play a role in disease transmission (Gottschalk, 2014). Poor ventilation, stress from overcrowding and poor farm management (mixing, moving, tattooing or weighing) are all risk factors for clinical cases. *S. suis* can survive in dust and feces in the environment.

The primary mode of prevention of *S. suis* is to alter management practices to minimize stress. Pigs should have adequate space allowances per head, calucualted based on group size, pen size and bodyweight. Poor ventilation sites and overcrowding should be avoided to decrease the chance of droplet transmission via the respiratory route. Farm management should be focused on biosecurity, cleaning and disinfection, temperature control, good air ventilation and rodent and pest control etc.

S. suis infection is increased in early-weaned pigs (Amass, Clark and Wu, 1995; Amass *et al.*, 1996; Baele *et al.*, 2001). In post-weaned pigs, maternal immunity is insufficient to prevent all the piglets from becoming infected. When piglets are moved to the nursery, infection can spread until the pigs produce sufficient immunity. In the critical post-weaning period, management should be targeted to prevent the outbreak and stress management is key.

Prevention of other disease that predispose infection by *S. suis*, including PRRS is also needed. Backyard pigs in Thailand generally lack appropriate biosecurity, often importing pigs or piglets from unknown sources and raising pigs of multiple age groups together. The morbidity and mortality rates for *S. suis* infection varies, but for pigs co-infected with PRRS, morbidity and mortality rates are much higher (Thanawongnuwech *et al.*, 2000). PRRSV causes damage to alveolar macrophages causing post-weaning pigs (5-6 weeks of age) to be sensitive to secondary bacterial infections from *S. suis* and *Hemophilllus parasuis* (the primary cause of Gleasser's disease with incidence and clinical features like *S. suis* meningitis). Farms experiencing high infection should consider culling and restocking from a *S. suis* free source. Pigs that die of an unknown cause should never be sold or consumed.

Personal hygiene is the key to prevention of *S. suis* infections in humans who are dealing with pigs or pork meat; such as farm workers, butchers, food preparers, should be in good health status, with no wounds on their hands, and use protective equipment. Lastly, pork should be cooked thoroughly before consumption.

1.9.3.1 Community control program

Most outbreaks of *S. suis* in humans in Thailand between 1999-2011 occurred in catering venues, and all were linked to consumption of traditional raw pork dishes (Fongcom *et al.*, 2009; Takeuchi *et al.*, 2012; Teekakirikul and Wiwanitkit, 2003; Vilaichone, 2002). A participatory community approach undertaken during an outbreak in Phayao province (31 confirmed cases and 3 deaths) revealed the source of the outbreak to be consumption of a raw traditional pork dish at a funeral (Khumlar *et al.*, 2013). Housewives were responsible for cooking in the meals and the men, especially elders, favored the traditional raw spicy pork dish. This study showed that

younger people also consumed raw pork. Traditional raw pork dishes are normally served at celebrations and are served with alcoholic drinks. Within the study, the the community dictated social measures to prevent eating raw pork dishes at celebratory events, gaining agreement that party holders serving raw pork would be responsible for any *S. suis* outbreak. The celebration menu would be discussed by party holders with the village commission and the housewives, who help with catering. Risk communication materials including posters, flyers and stickers were distributed in the area. Six-months after the public health intervention, public health officers found that 90% of villagers had stopped eating raw pork and that only cooked pork was served at parties and celebrations. This model has been replicated in other villages which have repeated *S. suis* problems in Thailand.

1.10 *Streptococcus suis* in Thailand

1.10.1 Recognition of *S. suis* in Thailand

S. suis was first reported in Thailand since 1987. The first report described six *S. suis* cases in the central region between 1987-1992 (17% of purulent meningitis cases in Ramathibodhi hospital in Bangkok). The cases responded to treatment but were left with persistent hearing loss. Three cases had a history of contact with pigs. Most cases in Thailand came from the northern provinces of Lamphun, Chiang Mai, Phayao, Pitsanulok, Kampangphet and Pichit (**Error! Reference source not found.**).

Figure 1-2 Map of northern region Thailand showed upper northen provinces. Source: http://wikitravel.org

Three severe cases were reported in 1997 and one case each year between 1998 and 2000 (Leelarasamee *et al.*, 1997*;* Chotmongkol *et al*., 1999; Vilaichone, 2002). A hospital study of 12 cases of *S. suis* in Bangkok between 1997-2002 divided *S. suis* clinical signs into three categories: meningitis; meningitis with neurological signs and arthritis with myelitis and septicemia (Bureau of General Communicable Diseases, 2007) and showed that consumption, or contact with pork was a risk factor.

The number of *S. suis* human cases reported in Thailand, has been increasing since 1999, beginning with an outbreak of ten cases reported from Lamphun province in the northern region. All patients were male, aged between 40-49 years with a history

of consuming raw or semi-cooked pork and blood and drank alcohol. They exhibited fever, muscle pain, diarrhoea and hemorrhage, but did not develop meningitis, and all died (Fongcom *et al.*, 2001). Cases were suspected to be of meningococcal disease but were negative for *Neisseria meningitidis*. *Streptococcus viridans*, part of the normal flora in mouth (but which can be pathogenic) was isolated from eight of the ten cases. Two cases were found positive for an identical genotype of *Streptococcus suis* serotype 2 that confirmed that the pathogen was from the same origin. This outbreak was ascribed to *S. suis*, acquired from consumption of raw pork and blood.

The epidemiologists at Lamphun hospital suspected limitations in the hospital diagnosis of *S. suis* and a descriptive prospective observational study was conducted between July 2001 and July 2002. All *Streptococcus viridans* isolations were submitted for genotyping at Faculty of Medicine, Chiang Mai University and 19 of 28 *S. viridans* cases were confirmed as *S. suis* (67.86%). All cases presented with acute meningitis with fever headache and stiff neck and had a history of drinking and consumption of raw pork or blood 1 - 7 days before presenting with clinical signs. Case fatality was high, seven of the 19 *S. suis* cases died, a case fatality rate of 37%. In addition, there were 3 case of deafness and 1 case of hemiparesis.

In a retrospective study from 17 patients in 2002, working in a pig farm and eating raw or semi-cooked pork were risk factors of *S. suis* infection. Nine cases developed meningitis, four with endocarditis, two with septicemia, one each with pneumonia and peritonitis (Vilaichone, 2002).

It is likely that *S. suis* is underreported considering the limitations of hospital diagnosis and low awareness among healthcare practitioners. Since 2005, *S. suis* has

been included within the epidemiological surveillance system in Thailand, and *S. suis* incidence has been increasing each year.

In 2007, there was an outbreak of *S. suis* in a district of Phayao province, northern Thailand (Khadthasrima *et al.*, 2007a) of 31 confirmed cases with 16.1% fatality rate and incidence rate of 6.2 per 100,000 in general population. Raw pork consumption was identified as the source of the infection (Takeuchi *et al.*, 2012). In 2008, 230 *S. suis* cases, with a morbidity rate of 0.36 per 100,000 population. Most cases were from the northern region (Bureau of Epidemiology Thailand, 2013). In 2009, 158 cases were reported, with a morbidity rate of 0.25 per 100,000 population. Cases peaked in May 2009 and again cases came from the northern region (Bureau of Epidemiolgy Thailand, 2013). In 2011, 185 cases of *S. suis* were reported, accounting for morbidity and mortality rates of 0.29 and 0.02 per 100,000 population, respectively. Again, most cases occurred in northern Thailand and the risk factors were consumption of raw pork (Hmonpangtiam and Chuknam, 2011).

Despite the large case numbers of *S. suis* reported in Thailand since 2009, only 66 investigation reports were submitted to the Bureau of Epidemiology between 2011 and 2013 (Wongkamma *et al*., 2014). Of these, 61 were individual investigation reports, and five were outbreak investigation reports. From 66 investigations, 47 cases were ascribed to consumption of raw or semi-cooked pork dishes together with regular alcohol consumption. Other risk factors included handling pork meat and working on pig farms. Two cases arose from consumption of raw buffalo meat and consumption of meat of an unknown source. The highest incidence was in the 45-60 age group and 11.11% cases resulted in hearing loss.

1.10.2 *S. suis* control collaboration network in Thailand

Thai farmers are not commonly aware of *S. suis*. For disease prevention, surveillance and control needs to be in both humans and animals. In Thailand, a collaborative multilevel network has been established among related organizations for the control of *S. suis* (Table 1-4).

Table 1-4 Organisations involved in *S. suis* control

Level	Ministry of Health	Ministry of Agriculture	Ministry of Interior
National level	• Department of Disease Control • Department of Medical Science • Department of Health • Department of Medical Services	• Department of Livestock Development • National Institute of Animal Health	• Department of Local Administration
Regional and Provincial level	• Regional Disease Prevention Control • Provincial Health Office • Medical Science Center	• Regional Livestock Office • Provincial Livestock Office • Veterinary Research and Development Center	• Provincial Administration Organization
Local level	• District Public Health Office • Sub-district Health Promoting Hospital • Health volunteer worker	• District Veterinary Office • Veterinary volunteer worker	• Municipality • Sub-district Administration Organization • Sub-district Headman • Village Headman

An integrated policy for *S. suis* control needs to be a systematic collaboration among related organizations. There are three ministries involved in disease control; the Ministry of Health, Ministry of Agriculture and Cooperatives and the Ministry of Interior. The Ministry of Health (Department of Disease Control, Regional and Provincial Health Office and local hospitals), are responsible for surveillance, investigation, prevention and outbreak control. The Department of Health is

responsibile for public management of personal health and disease prevention to communities, sanitary control of meat in markets and gives knowledge to food handler and processor. The Ministry of Agriculture and Cooperatives, Department of Livestock Development and other organizations under their control, are responsible for surveillance, investigation and control of animal diseases as well as zoonoses. The Ministry of Interior, Department of Local Administration, is for responsible for funding and for collaboration with the other ministries. In terms of activities the following apply;

i) *S. suis* investigations and passive surveillance in humans are the responsibility of the surveillance rapid response team (SRRT), mandated to investigate, report and exchange information with the animal sector at central to local level;

ii) Surveillance in animals, including both clinical laboratory surveillance and active surveillance is the responsibility of the Department of Livestock Development;

iii) The Department of Disease Control integrates all operations for control and prevention of *S. suis* infections in humans and animals (from animal production to consumer behavior);

iv) Health education and public relations to provide knowledge to the public and risk groups such as farmers, butchers, food processors and

consumers are within the mandate of all organisations. Knowledge of animal husbandry, slaughter procedures, and hygiene is essential.

1.10.3 *S. suis* research in Thailand

In Thailand *S. suis* in pigs has been studied on farms and in slaughterhouses, mostly in the northern region where there is a high incidence of *S. suis* infection in humans. A slaughterhouse study in five provinces in the northern region found a prevalence of 15.77% (95%CI 4.44-18.06%) for *S. suis* (Lakkitjaroen *et al.*, 2009). A study of *S. suis* in pigs in Chiang Mai Province showed an overall prevalence of 9% (n=212) with prevalence being significantly higher in districts located at a greater distance, south of Chiang Mai city. *S. suis* serotype 2 presented more in healthy pigs (43%) than in sick pigs (10%) (Padungtod *et al.*, 2010).

During an outbreak in Phayao province, in the northern region, where there was an outbreak of 31 confirmed-cases in 2007, the prevalence of *S. suis* in slaughtered pigs was 73.33% and of these 7.78% were serotype 2 (Neungmek and Pathanasophon, 2011). A survey in the western and eastern region of Thailand, also found a very high prevalence in piglets and fattening pigs of 33.4% and 35.6% respectively (Pathanasophon *et al.*, 2009).

From the few genetic profile studies that have been undertaken from *S. suis* isolated from human cases in Thailand show ST1 as the major multilocus sequence type (ST). Type ST27 and ST104 were also identified (Takamatsu *et al.*, 2008; Kerdsin *et al.*, 2009, 2011a). A study reported in 2006 indicated a large genetic diversity of *S. suis* isolates among humans and pigs indicating transmission of *S. suis* from pigs to humans (Wongsawan *et al.*, 2006).

1.11 Research design

The research described in this study aimed to estimate the epidemiology and burden of *S. suis* in Chiang Mai to optimize prevention and control and inform policy and to province in the Northern region of Thailand, Specific objectives included i) characterising the epidemiology of human *S. suis* cases; ii) estimating the economic burden of *S. suis* in Chiang Mai; iii) estimating the prevalence of *S. suis* in pigs, iv) characterization of the pig production system and examination of risk factors for *S. suis* in pigs and v) chacterisation of circulating serotypes of *S. suis* to understand the population biology of *S. suis* in Chiang Mai province. The mixed methods research design of this study included both quantitative participatory approaches and qualitative approaches, including cross sectional surveys.

1.11.1 Cross-sectional study

In a cross-sectional study the defined population is only assessed once at a point of time. A descriptive cross-sectional study design is applied to assess the baseline characteristics of the population. The sample is examined individually for the presence or absence of the disease so that the prevalence, risk factor and the outcome of intervention and control measure can be determined (Broeck and Brestoff, 2013; Thrusfield, 2007). In a cross-sectional study, those sampled should be representative of the entire population under study which involves the calculation of a number of samples from a population or a representative subset and sampling methods (Broeck and Brestoff, 2013). The best approaches use various random sampling strategies to select to the study group, including simple random, systematic, stratified, cluster and multistage sampling (Pfeiffer, 2010; Silva, 1999).

A cross-sectional survey has several advantages over other observational study designs (Broeck and Brestoff, 2013); it is relatively quick and easy to perform (Mann, 2003; Pfeiffer, 2010); it is moderately cheap as multiple outcomes can be studied at a particular time and requires no follow-up (Mann, 2003); it can determine the prevalence of risk factors and the frequency of cases of disease in a defined population and it is useful for measuring current health status (Bailey, 2005; Silva, 1999). A disadvantage of cross-sectional studies is that they are not suitable to investigate rare diseases or diseases of short duration since it is rare to find the disease case (Silva and International Agency for Research on Cancer, 1999). Any difficulty in re-calling past events may also contribute bias (Pfeiffer, 2010).

1.11.2 Qualitative

Interviews are one of the most common strategies to access people's experiences and perceptions, attitudes, and feelings through simply asking questions and getting answers from participants involved in a study. Qualitative research can be conducted as an in-depth interview with a single respondent, a pair of individuals, a small group or larger group discussion. Research interviews are commonly categorized in three fundamental types that are structured, semi-structured and unstructured (DiCicco-Bloom and Crabtree, 2006).

Structured interviews aim to gather data from large samples to ensure consistency of response, and often generate quantitative data. However, they can also be used as a qualitatively. They are easy and efficient to administer. In structured interviews, the interviewer asks each respondent a set of predefined questions. Although it allows the interviewer to clarify the questions to the interviewee in case

they find them confusing but this should be without variation or possibility for follow-up question, therefore it is limited participant response.

The unstructured interview can be defined as an interview for which neither the questions nor answer categories are predetermined (Minichiello *et al.*, 1992). They are an informal, flexible and free flowing conversation. This approach is suitable for an interview that needs depth and sensitive information. The unstructured interview may begin by asking relatively open-ended question and more questions will be generated in response to the interviewee's narration (Zhang and Wildemuth, 2009). An unstructured interview allows exploration of deep information with further questions and topic areas which the interviewer see as significant without the limitation of pre-set questions. This method is time consuming and since there are no prearranged questions it is difficult for interviewers to have control over the direction and pace of the conversation. It is difficult to control the degree of directedness of the questions and statements proposed during the conversation on (Zhang and Wildemuth, 2009).

The semi-structured interview approach, applied in this study is used most frequently in healthcare, as it provides participants with some guidance and direction for the conversation (Gill *et al.*, 2008). It is conducted within a fairly open framework, which allows the participant to respond in more detail, but the topics and questions are prepared in advance. It is more flexible than a structured interview, allowing the participants to reveal information they think is important but has not been considered by the research team. This type of interview requires a good sample of interviewees to generate sufficient information for comparison.

1.12 Thesis structure

This thesis comprises four data chapters. Chapters 2 and 3 concentrate on the epidemiology and disease burden of *S. suis* in humans. Chapters 4 and 5 examine the epidemiology and risk factors influencing transmission of *S. suis* in pigs.

Chapter 2 presents an analysis of human *S. suis* surveillance data in Thailand, providing a detailed profile *S. suis* transmission. Analysis of human *S. suis* surveillance data collected between 2005-2014 in Chiang Mai was used to characterize the epidemiological characteristics of human *S. suis*. Chapter 3 examines the overall disease burden of *S. suis* in Chiang Mai province by using Disability Adjusted Life Year (DALY) and includes an estimation of the social and economic burden of *S. suis*.

Chapter 4 presents the results from a cross-sectional study estimating the prevalence of *S. suis* in backyard pigs and examining risk factors associated with production and management practices. Chapter 5 examines the population biology of *S. suis* in backyard pigs by genetic characterization of circulating strains.

Chapter 6 highlights the conclusions and recommendations from the study.

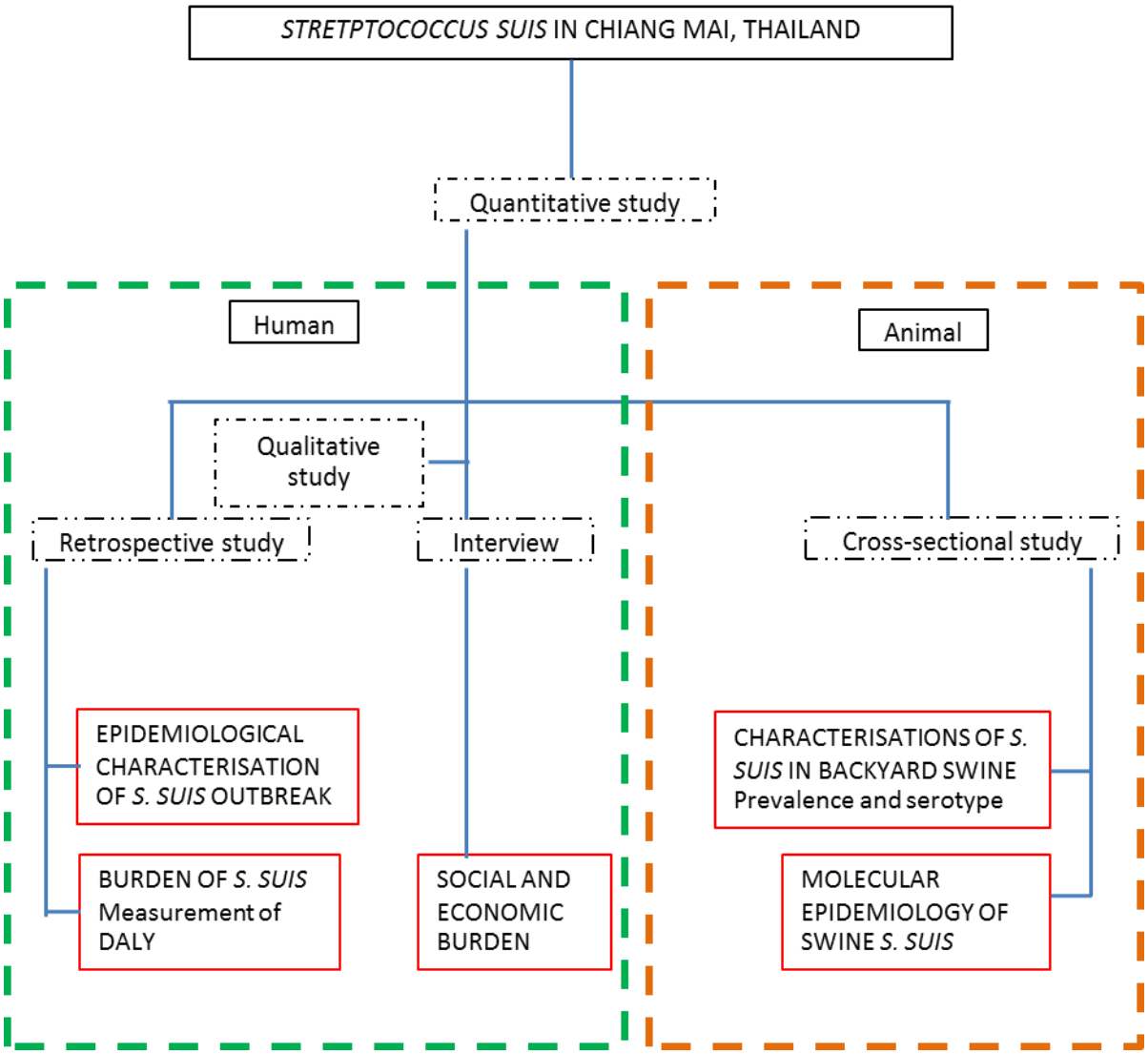


Figure 1-3 Conceptual model of the research and study components

CHAPTER 2-Human *Streptococcus suis* in Chiang Mai, Thailand

2.1 Study aims

This study analyses the human *Streptococcus suis* surveillance data collected between 2005 and 2014 in Thailand to characterize the epidemiological characteristics of human *S. suis* over this period.

2.2 Introduction

S. suis is a zoonotic bacterial pathogen that has a reservoir in pigs but can cause disease in humans (Gottschalk, 2014). In humans, infection can cause moderate to severe disease and can result in death or disability (Donsakul *et al.*, 2003; Wertheim *et al.*, 2009a). Consumption or exposure to infected pig and pork products are the main risk factors (Ho *et al.*, 2011; Neungmek and Pathanasophon, 2011). While the first case of *S. suis* infection in Thailand was reported in 1987, it was not until a large outbreak of 29 cases in Phayao province in 2007, that there was general public awareness of the pathogen (Takeuchi *et al.*, 2012).

Outbreak investigations have shown that *S. suis* in Thailand occurs most frequently in the northern provinces (Gottschalk *et al.*, 2014; Takeuchi *et al.*, 2012; Thayawiwat, Wichaikham and Painpringam, 2013; Wongsawan *et al.*, 2006). Outbreaks of *S. suis* in Thailand are associated with consumption of pork and are linked to food served during festivities. *S. suis* is increasingly recognized as a bacterial pathogen that causes hearing loss and death in Thai people who eat raw or undercooked pork. The public health authority in the northern region, developed a surveillance system for *S. suis* that has been adopted in hospitals since 2005. Records are sent to the Bureau of Disease Control and *S. suis* has been included in the national surveillance system since 2009.

This chapter retrospectively examines surveillance data for *S. suis* in humans in Chiang Mai province collected between 2005 to 2014 and describes the *S. suis* surveillance system and food safety policy systems in Thailand.

2.2.1 Profile of Thailand

2.2.1.1 Location

Thailand is situated between latitude 5º 37' and 20º 27', longitudes 97º 22' N and 105º 37' E, covering an area of 513,120 km^2 (Figure 2-1). Thailand lies at the center of the Indochina peninsula in South East Asia, bordered by Myanmar to the north and west, Laos PDR to north and east, Cambodia to the southeast. Its southern province occupies the spine of the Malaya peninsula, where Malaysia borders it in the south. Thailand is a member of the 11 Southeast Asian Nations that includes; Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Timor-Leste, Thailand and Vietnam.

The National Research Council divides 77 provinces of Thailand into six geographical regions, based on natural features. The northern and western regions are high and mountainous with steep river valleys. The northeastern region is a dry plateau which in places is flat, with a few low but rugged and rocky hills. The central region is a plain with natural self-contained basin. The eastern region is consists of short mountain ranges alternating with small basins of short rivers that drain into the Gulf of Thailand. The southern region is rolling and mountainous terrain with access to Andaman Sea and the Gulf of Thailand (Figure 2-2).

Figure 2-1 Map of Southeast Asia showing Thailand; Source: http://www.thailand-maps.com

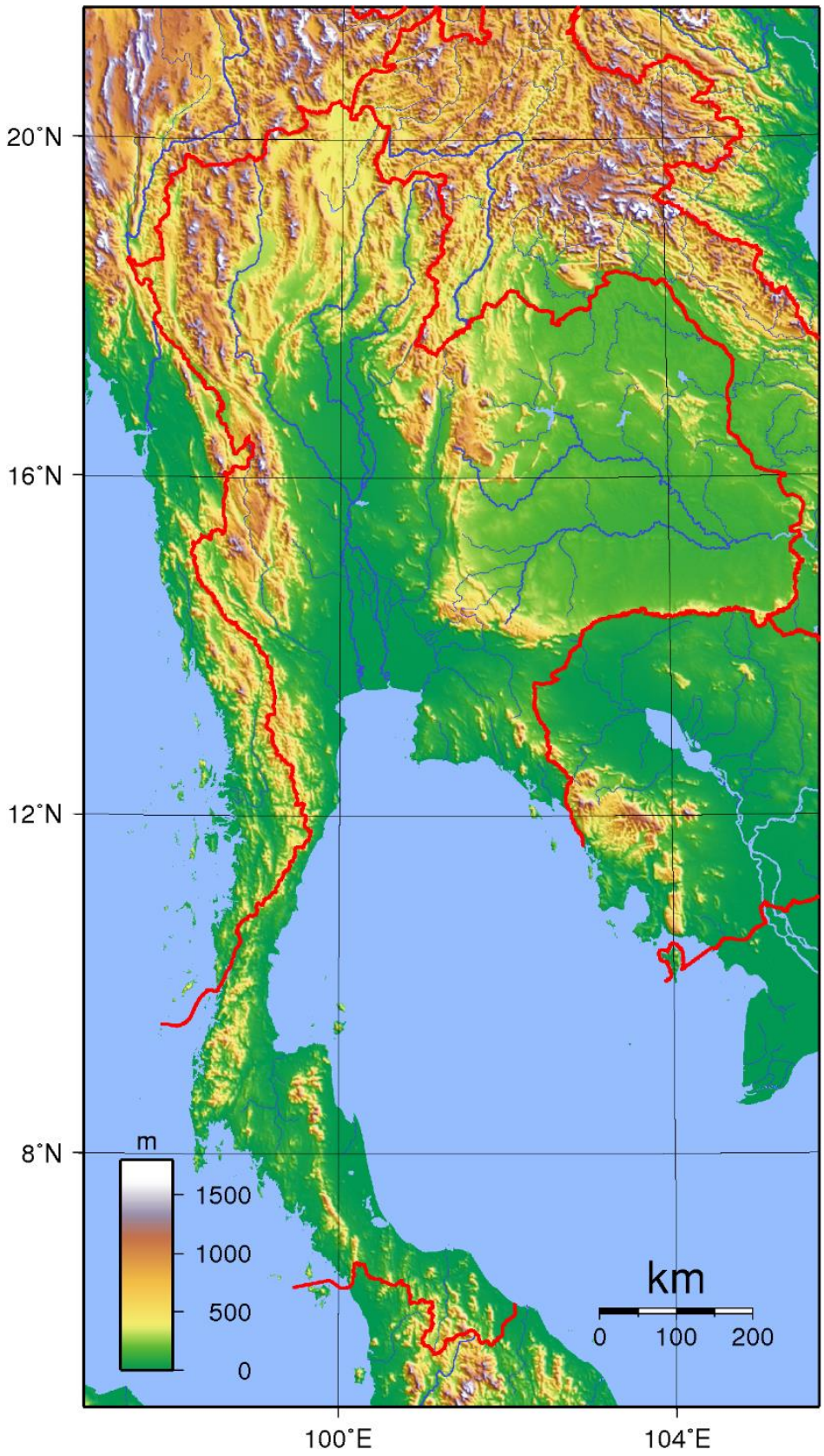


Figure 2-2 Topographic map of Thailand; Source: http://commons.wikimedia.org

2.2.2 Profile of Chiang Mai

2.2.2.1 Location

Chiang Mai is the largest province in the northern region of Thailand (Figure 2-3). It is located on the Ping river basin with mountainous terrain. The Chiang Mai valley is 310 meters above sea level covers an area of 20,107 km^2. The province measures 136 km at its widest and 320 km at its longest. Chiang Mai is covered by mountains and rain forest hills. The highly mountainous area is 500 meters above sea level, surrounded Chiang Mai to the north and west and covering 80% of the province. The mountains are incised by steep river valleys and upland areas, suited to agriculture.

2.2.2.2 Administrative system and culture

Twenty-five districts of Chiang Mai, are subdivided into 204 sub-districts, further subdivided into 2,066 villages. Chiang Mai has a population of 1,687,000. Most of the population is Thai, but a variety of minority hill tribe groups such as Karen, Meo, Yao, Laha, Lisu, Akha and Lua inhabit the region (http://www.chiangmai.go.th).

2.2.2.3 Climate

Chiang Mai has a tropical wet and dry climate, with the average temperature s of 25.4^{o}C. The climate is controlled by tropical monsoons across three seasons. Summer extends from March through May, and is hot and humid with high temperatures above 30^{o}C. The rainy season occurs from May through October with frequent rains and thunder showers, and an average rainfall of 1,100-1,200 millimeters. The winter season runs from November through February, with the average temperatures of around 20^{o}C.

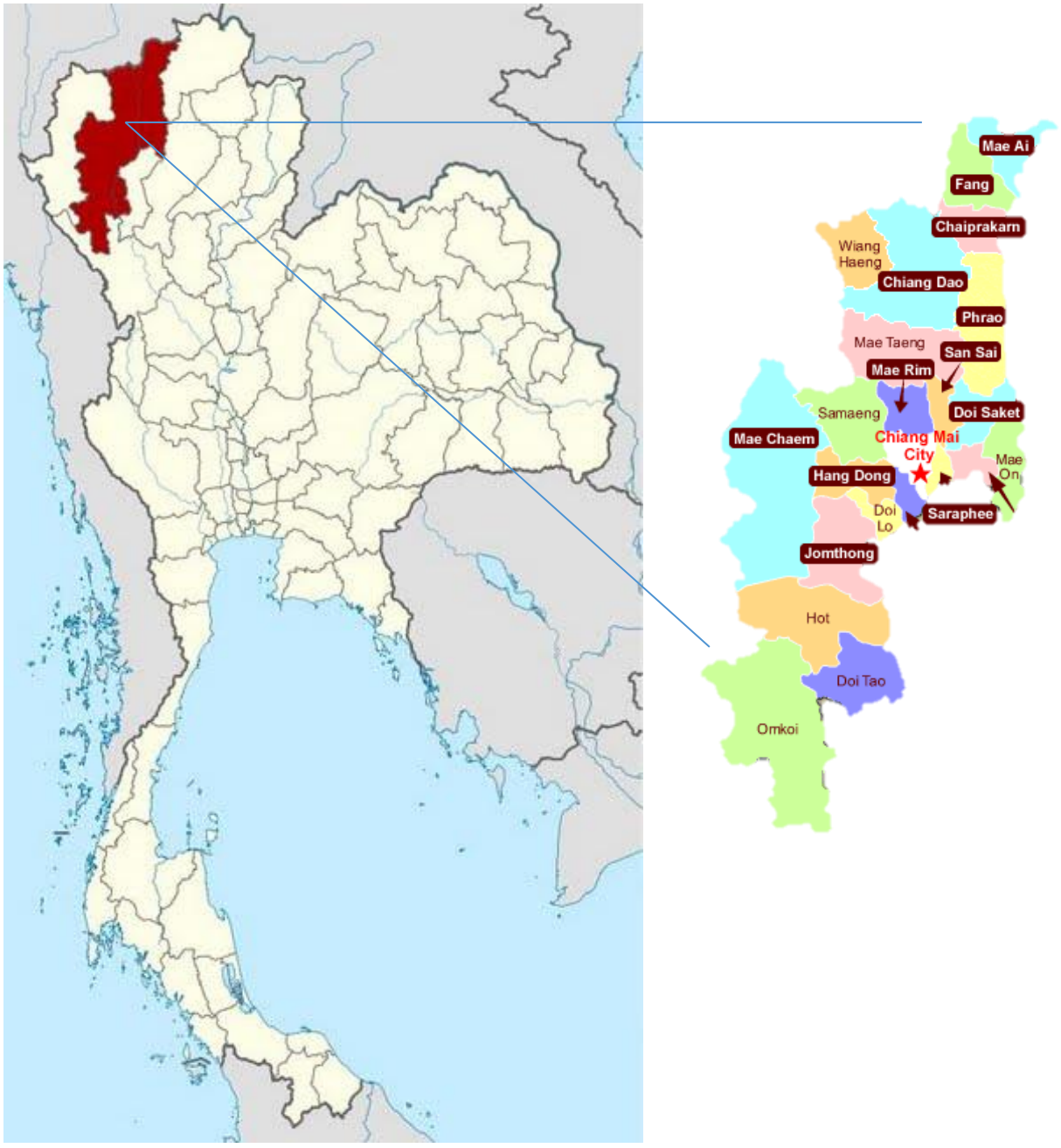


Figure 2-3 Map of Thailand showed Chiang Mai province and its districts. Source: https://commons.wikimedia.org; https://thaimission.com

2.2.3 Disease surveillance

The term "surveillance" is defined in the dictionary as the "close and continuous observation of one or more persons for the purpose of direction, supervision, or control (Choi, 2012). Disease surveillance refers to the ongoing systematic collection, analysis, and interpretation of data, closely integrated with the timely dissemination of these data to those responsible for preventing and controlling disease and injury (Thacker and Berkelman, 1988).

2.2.3.1 History of Disease surveillance

The first public health action that used morbidity and mortality data, dates back to the fourteenth century. Surveillance was based on individual contacts of infectious patients. The Venetian Republic appointed three guardians of public health to detect and exclude ships that had infected people aboard during the occurrence of Black Death or pneumonic plague in 1348 (Declich and Carter, 1994).

Surveillance was first applied for management of public health by John Graunt from 1662. Graunt disseminated the death statistics for the city of London in a weekly "Bill of Mortality", that were collected from 1532. Gaunt was the first to use these data to quantify the pattern of disease and study the cause of disease.

The first fully developed surveillance system to direct public health was in the nineteenth century, involving the collection and interpretation of health-related data; sex, age, occupation (Declich and Carter, 1994). The systematic reporting of various diseases began in the United States in 1874, followed by Italy in 1888 and the United Kingdom in 1890 (Chorba *et al.*, 1989).

At International level, public health surveillance is coordinated by the World Health Organization (WHO). The 21st World Health Assembly established surveillance as an essential function of public health practice (Choi, 2012).

The term surveillance was used initially in public health to describe the close monitoring of persons who, because of exposure, were at risk for developing highly contagious and virulent infectious disease (Teutsch and Churchill, 2000). In 1963 Alexander D. Langmuir defined the term Surveillance as "the continued watchfulness over the distribution and trends of incidence through the systematic collection, consolidation and evaluation of morbidity and mortality reports and other relevant data and the regular dissemination of data to all who need to know" (Thacker and Berkelman, 1988). The 21st World Health Assembly in 1968, the assembly expanded Langmuir's definition to include the assumption that surveillance information is collected in order to take appropriate action to improve health outcome (Choi and Choi, 2012). At present, the definition of public health surveillance given by WHO is the continuous, systematic collection, analysis and interpretation of health-related data needed for the planning, implementation, and evaluation of public health practice. Surveillance can: (i) serve as an early warning system for impending public health emergencies; (ii) document the impact of an intervention, or track progress towards specified goals; and (ii) monitor and clarify the epidemiology of health problems, to allow priorities to be set and to inform public health policy and strategies (Anon, n.d.).

2.2.3.2 National public health surveillance of Thailand

To control the international spread of diseases, the World Health Assembly in 1969 developed the International Health Regulations (IHR) "to ensure the maximum security against the international spread of diseases with a minimum interference with

world traffic" (World Health Organisation, 1983). The regulations were adopted and by all WHO member states. Thailand, adopted the IHR regulations and revised these into several Acts and Regulations including the Communicable Disease Act (B.E.2523), the Public Health Service Act (B.E. 2535) and the Zoonosis Act (B.E..2499) (Boonchoo, 2012). As a result, Thailand as other member states, had to develop the public health surveillance system and emergency response for both national and international level. This was an opportunity for Thailand to improve the public health and surveillance control system in according to the international standard. The latest IHR in 2005, purposed "to prevent, protect against, control and provide a public health response to the international spread of disease in ways that are commensurate with and restricted to public health risks, and which avoid unnecessary interference with international traffic and trade". This aimed to provide security against the international spread of epidemic disease with a minimum interface with world traffic. They require that: each nation health administration should inform WHO within the first 24 hours of being notified of the first suspected case on its territory of a disease subject to the Regulations. All subsequent cases and deaths should be reported to WHO (World Health Organization, 2008).

Thailand established national public health disease surveillance in 1970, in agreement with WHO. At first, 14 fatal contagious diseases were registered (Areechokchai, 2014). Today, national surveillance covers 84 reportable diseases, including *S. suis* (Bureau of Epidemiology, 2006). The epidemiological surveillance network in Thailand includes all related healthcare services, both private and public in the communities, the provincial public health offices in the provinces and uses the Bureau of Epidemiology as the national reporting center.

2.2.4 Food-borne diseases

There are ten notifiable foodborne diseases in Thailand; hepatitis, enteric fever, dysentery, cholera, food poisoning, mushroom poisoning, acute diarrhea, trichinosis, *S. Suis* and brucellosis (Bureau of Epidemiology, n.d.). Efforts to improve standards of food hygiene in Thailand, between 2004-2013 have led to a decrease in the incidence of foodborne disease (www.boe.moph.go.th). However, in 2015 the number of cases of cholera and *S. suis* increased. Cholera from 0.02 to 0.05/100,000 population and *S. suis* from 0.29 to 0.50/100,000 population from 2014 - 2015. Most cases of foodborne infection were reported from the northern region.

2.2.5 Food safety policy

Over the past three decades, consumers in Thailand have become increasingly perceptive regarding food safety, although their main concerns are chemical residues in food products. As concerns related to food safety have increased, the government has established systems and regulations to regulate the safety of agricultural products and foods (The Thailand Food Committee, 2012). Thailand has a strong food safety policy under the National Food Committee Act 2008, chaired by the Prime Minister. The secretary and co-secretary of the committee are mandated by the Secretary General of Food and Drug Administration, under the Ministry of Public Health and Co-secretary of National Bureau of Agricultural Commodity and Food Standards, under the Ministry of Agriculture and Cooperatives. Many organisations are involved including; Ministry of Public Health, Ministry of Agriculture and Cooperatives by the National Bureau of Agriculture Commodity and Food Standards, and other organization in the food supply chain (Figure 2-4) (Anon, 2008).

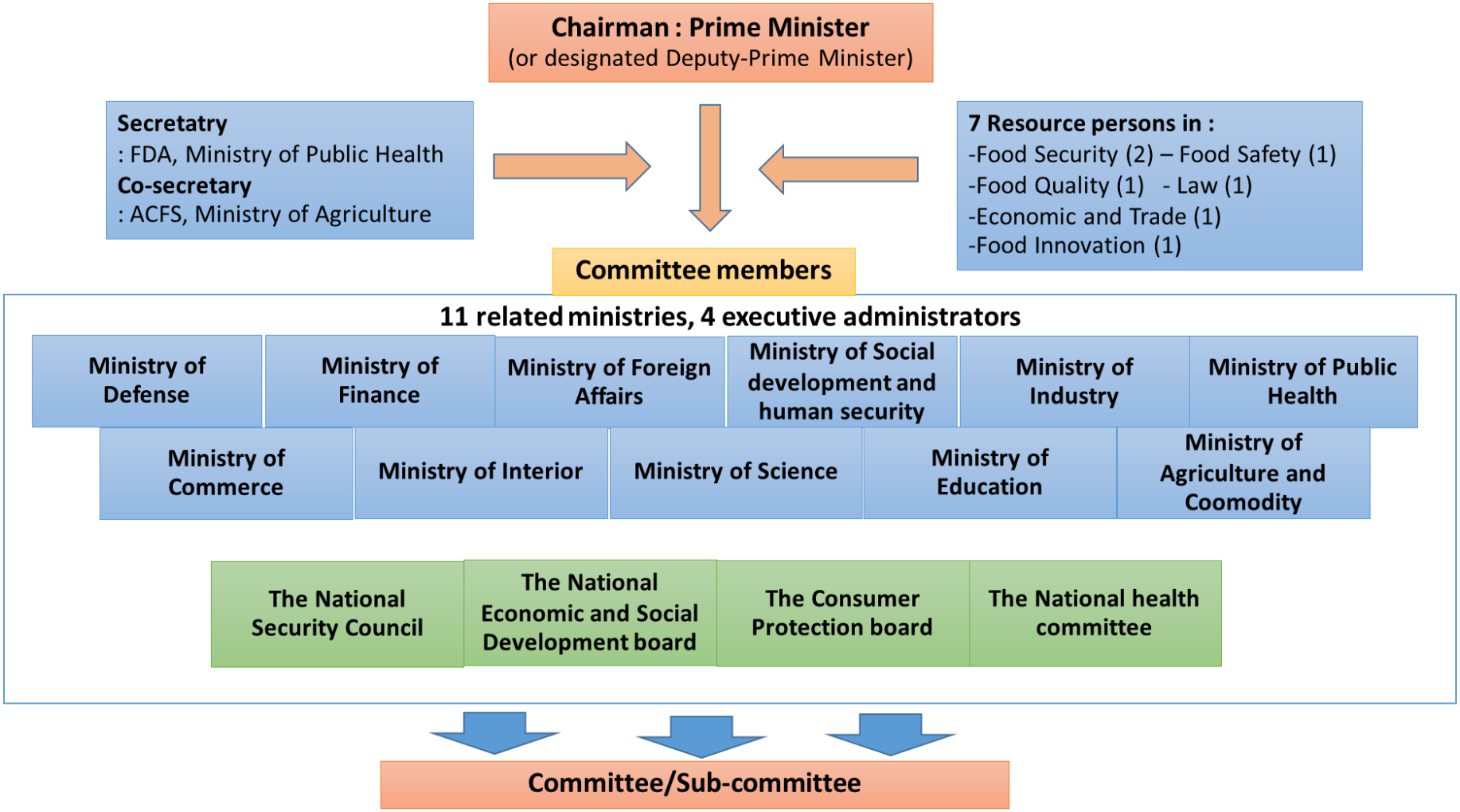


Figure 2-4 Food safety structure: The National Food Committee compoment; Source: Strategic Framework for Food Management in Thailand

2.2.6 Strategic Framework for Food Management in Thailand.

Thailand aims to produce food of a good quality standard that is safe food both for Thai people and for export globally (The Thailand Food Committee, 2012). Strategies to accomplish this vision include: i) Increasing the efficiency of resource management for sustainable national food production; ii) Ensuring food products from households, the community and industry level are of a good standard, conform to food safety and have high levels of nutrition; iii) Create food education and research systems that deliver a body of knowledge in all aspects of food production, including knowledge distribution to interested agencies; iv) Improve the efficiency of the food management system including food related law and information, and v) To create food security in households and communities in normal and emergency circumstances.

The four strategic themes to achieve these goals are: 1) Food security; 2) Food quality and food safety; 3) Food education and 4) Food management (The Thailand Food Committee, 2012).

The activities implemented for food quality and safety in Thailand to strengthen food production at local level and industry level are shown below (Figure 2-5).

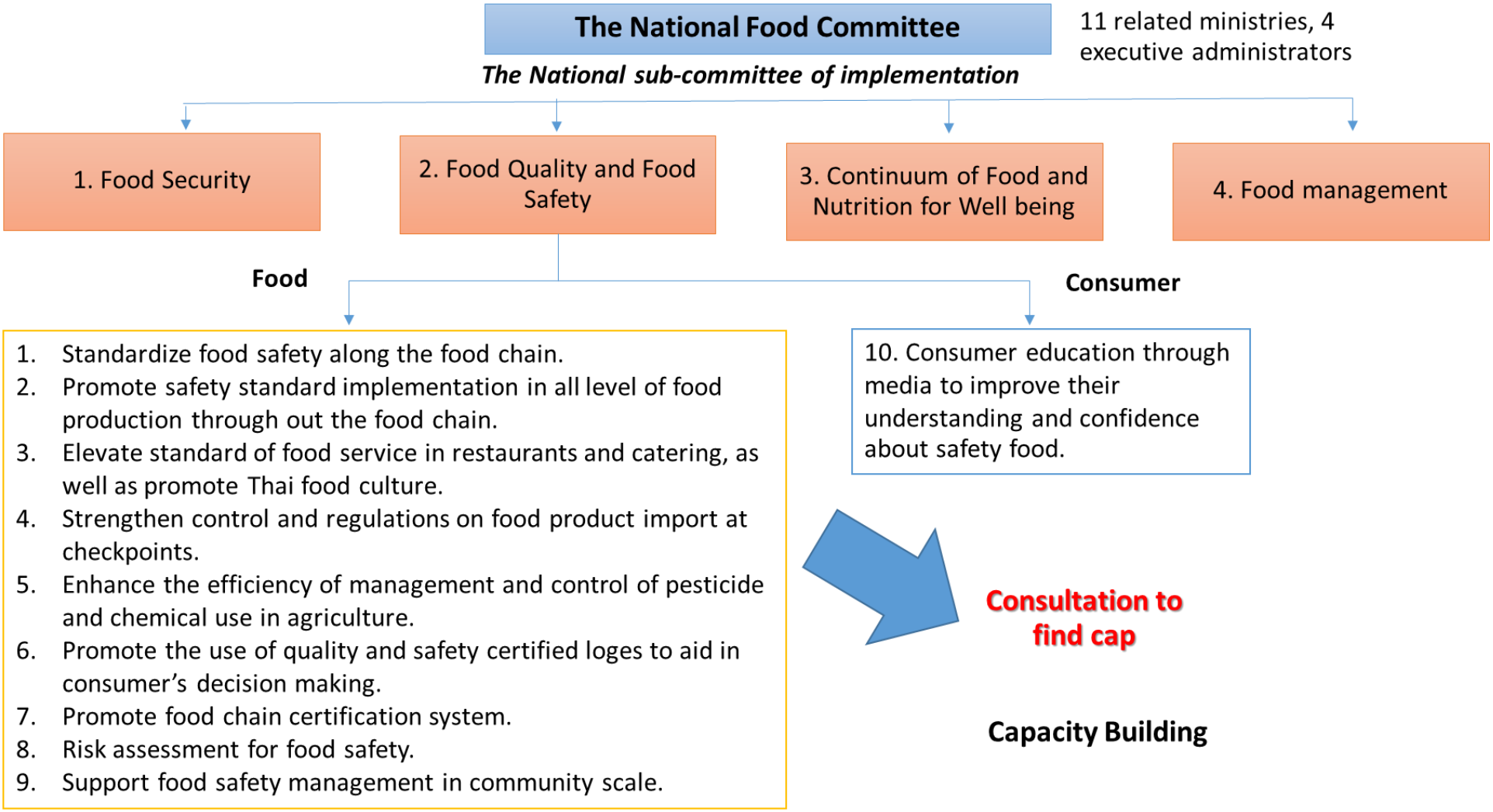


Figure 2-5 Strategic theme for food quality and food safety; Source: Strategic Framework for Food Management in Thailand

2.2.7 Meat hygiene

Many zoonoses can pass from animals to humans from consumption of contaminated meat. This increases consumer concern as to the origins of the meat products they consume. Government and industry organizations in Thailand have realised the importance of food safety. The Department of Livestock Development is protecting consumer health by enforcing access to safe and wholesale animal products, applying measures throughout the production chain. These include farming systems as well as legislation or rules to underpin actions to achieve food safety such as the Food Act (B.E. 2522), the Agricultural Standards Act (B.E. 2551), the Animal Slaughter Control and Meat Sale Act (B.E. 2551) and other acts, regulations, proclamations and orders of Department of Livestock Development (Anon, 2014a). The quality assurance sign showing the letter Q awarded by Department of Livestock Development is the certificate for the butchers passing the Department's standards on farms, slaughterhouses and for meat processing. In 2015, there were 9,000 animal farms, 1,874 slaughterhouses and 4,000 meat markets whose hygiene standards were certified by the Department of Livestock Development.

2.2.8 Surveillance system under One Health concept

The development of surveillance and control systems under the "One Health" concept is one of the strategies in the Thailand Strategy Plan for Prevention and Control of Emerging Diseases 2013-2016, aiming to reduce morbidity, mortality, and reduce economic, social, and environmental impact due to emerging diseases (Kitpati, n.d.). It has been estimated that 75% of emerging diseases in humans are zoonotic (Taylor *et al*., 2001). Over decades, ecosystem changes occurring as a natural

phenomenon or through human intervention have disrupted the ecological balance. Perturbations in habitat that can lead to changes in distribution of reservoir hosts and their biodiversity, can play an important role in regulating the transmission and emergence of infectious disease (Patz *et al.*, 2005). A One Health framework for working together to address emerging threats to health and food security has been developed in Thailand.

2.2.8.1 One Health in Thailand

Thailand has implemented surveillance, prevention and control systems that are underpinned by the One Health concept, establishing networks among multi-sectoral organisations including government, state enterprises and the private sector. The primary collaboration involves the Ministry of Agricultural and Cooperatives, the Ministry of Public Health, and the Ministry of Natural Resources and Environment (Kitpati, n.d.). These organisations work together to develop and implement policies that increase collaboration in surveillance and control of emerging diseases.

One Health activities are in collaboration with Bureau of Disease Control, Department of Livestock Development, Department of National Parks, Wildlife and Plant Conservation and the Zoological Park organization (Kitpati, n.d.). and include surveillance for early detection of zoonotic EIDs in human and wildlife and training the trainers training for one health epidemiologists in the provinces and districts; strengthening emerging disease surveillance and response.

2.2.9 National surveillance for *S. suis* in Thailand

S. suis is a notifiable zoonotic disease in Thailand, important especially in the upper northern part of the country, where people commonly eat traditional raw pork

dishes (Fongcom *et al.*, 2001; Wertheim *et al.*, 2009b). Several large outbreaks in humans have occurred in this area and local public health officials have developed a surveillance system to survey and control *S. suis* in this region that has been expanded to the whole of Thailand.

The pathway for detection of *S. suis* detection and reporting is shown in Figure 1-1. First, *S. suis* cases are identified based on presentation of clinical signs together with a history of pig contact or consumtion of a pork product, especially ingestion of raw pork traditional dishes. Second, a sample from suspected patients is collected and the infection confirmed by laboratory isolation of *S. suis*. Once a case of *S. suis* is confirmed, it is reported to the Provincial Health Office using the communicable disease form (Report 506). Finally, the Surveillance Rapid Response Team (SRRT) will be dispatched to confirm the outbreak, identify and investigate new cases and guide prevention efforts. The SRRT operates at five levels; local community level, primary public health response level, intermediate public health response level, regional public health level and at National public health response level (Salit-apiruk, 2012). Cases are reported weekly by the Provincial Health Office to the Bureau of Epidemiology and surveillance data are published weekly, monthly and annually.

2.3 Methodology

2.3.1 Study method

To characterise the epidemiological characteristics of human *S. suis* cases in Chiang Mai, a 10-year retrospective review of human *S. suis* cases in Chiang Mai Province was undertaken.

2.3.2 Data collection

S. suis is a notifiable disease in Thailand. All cases are reported to the Provincial Health Office, Disease Prevention Control Office, and Department of Disease Control. In this study, *S. suis* cases were reported to Chiang Mai Provincial Health Office, Ministry of Public Health, Thailand between 2005-2014. Case reports were used to characterise *S. suis* epidemiology according to person, time and place.

2.3.2.1 Case reports

S. suis case reports describe the age, sex, nationality, address, onset date, admission and discharge date for the case.

2.3.2.2 Demographic data

Demographic data and human population data (annual) were obtained from the National Statistics Office (www.nso.go.th). District level population and annual age and sex stratified population data were obtained from Department of Provincial Administration, Ministry of Interior (www.dopa.go.th).

2.3.3 Data analysis

2.3.3.1 General principles

Analyses were based on reported cases to Chiang Mai Provincial Health Office between 2005-2014. The month variable used in analyses was based on the date of onset of disease, obtained from the case reported by the healthcare officer (general practitioner, nurse or epidemiology officer).

2.3.3.2 Population data

Population data was obtained from National Statistics Office. Total population data for Chiang Mai province and for Thailand per year were available and average populations for each Chiang Mai district were accessed. To calculate age and gender specific rates, data were aggregated into the following age categories for the analyses*:* 0-10, 10-20, 21-30, 31-40, 41-50, 51-60 and ≥ 60 years.

2.3.3.3 Data analysis

Descriptive epidemiology was applied to analysis of the data. Descriptive epidemiology is a method for comprehending the health status of a population, to make hypotheses about the cause of disease, and to inform program planning and valuation (Aschengrau and Seage, 2014). Disease patterns are analysed by classified the occurrence of disease according to variables of time, place and person; time refers to when and over what period the condition has occurred; place refers to where the condition occurs; and person refers to the affected persons (Friis, 2010).

2.3.3.4. Time variables

The occurrence of disease changes frequently and is unpredictable over time. It is beneficial to identify the characteristic of the disease when there are changes of incidence rate, or seasonal effects. Time variables are useful to determine the source of the disease whether it is a point source of infection, continuing common source, or intermittent exposure (Thomas and Weber, 2001). Time data are usually displayed with a two-dimensional graph. The number or rate of cases is plotted over time; the y-axis usually shows the number or rate of cases and the x-axis shows time periods such as years, months, or days, displayed as line graphs or histogram (Spellman and Stoudt, 2013).

2.3.3.5. Place variable

Health events vary according to place. Characterising the occurrence of disease by place provides insights into the geographical difference or extent of the problem**.** Place can be any geographic area where it is suitable to describe the occurrence of disease; such as country, district, place of residence, place of birth or employment, or as small as the hospital unit or place of diagnostic (Gregg, 2008). Sometimes, place can refer to the localized occurrences of disease such as the area associated with specific environment condition in a particular geographic area (Friis, 2010). Analysing data by place can help to identify the source of disease and mode of transmission or can help generate hypotheses about the source**.**

2.3.3.5. Person variable

Sociodemographic and behavioral characteristics of individuals may affect the occurrence of disease**.** Person characteristics that are associated with health include: inherent characteristic (age, sex, ethnicity); biological characteristics (immunity, nutrition); socioeconomic condition (education, occupation, housing, access to medical care); acquired characteristics (marital status), or health related, beliefs, and behaviors (use of medication, tobacco, alcohol consumption)**.**

Age and sex are the most common variable to analyse. Age is the most important attribute for person variable since age can involve to most of the health**-**related event; such as susceptibility, opportunity for exposure, latency or incubation period of the disease, and physiologic response (CDC, 2006). Another important attribute variable is sex which is related to the difference in occurrence of some diseases. Sex differences include: genetic, hormonal, anatomical, or other inherent

differences between sexes which may affect the susceptibility or level of exposure, resulting in presenting of some diseases (CDC, 2006).

2.3.4 Presentation of analyses

The descriptive epidemiology was set out as summary tables and supplementary figures, describing overall epidemiology at provincial level. These included reported cases from 2005-2014; the occurrence by month, by age and by gender-specific rates. Additional graphs and figures were used where necessary to illustrate other important aspects of the disease epidemiology.

2.3.4.1 Aspects of descriptive epidemiology

Descriptive epidemiology was used to define the surveillance data to describe epidemiological characteristics of *S. suis* for Chiang Mai province overall examining: the trend in *S. suis* incidence over time; the geographic distribution of *S. suis* and the demographic distribution of *S. suis.*

Trends in reported number of cases

The incidence and fatality rates for *S. suis* cases, by year between 2005-2014 in Chiang Mai province were calculated. Incidence rates were given per 1,000,000 population (number of reported cases divided by the population for that year multiplied by 1,000,0000). The number of cases per month between 2005-2014, were calculated.

Frequency of *S. suis*

The health outcome varies among populations, geographical areas and over time. Epidemiological studies quantify the frequency of health outcomes as the number of occurrences in a defined population over a defined time-period.

Incidence rate

S. suis cases were described using the incidence rate **(*I*)** to measure the rapidity with which new cases of disease develop over time. Incidence rate is the frequency of new cases in a defined population at risk during a specific time-period. It is also known as person-time incidence rate**.** It is calculated using the following formula.

$$I = \frac{number\ of\ new\ cases\ of\ disease\ that\ occur\ in\ a\ population\ during\ each\ year\ of\ study\ period}{the\ sum\ overall\ individual\ \ at\ risk\ of\ developing\ disease\ of\ that\ year}$$

Case fatality rate

The case fatality rate (*CF*) also called the case-fatality ratio, is the proportion of persons with a particular condition (individual diseased humans) who die from that condition. The case fatality rate is calculated using the following formula:

$$CF = \frac{number\ of\ death\ during\ each\ year\ of\ study\ period}{number\ of\ cases\ during\ that\ year}$$

2.3.4.2 Geographic distribution of human *S. suis*

The reported cases and incidence rates of human *S. suis* at district level and per 100,000 persons were calculated (number of reported cases in each district divided by the average population over ten-year period in that district multiplied by 100,000). Only those districts that had *S. suis* reported were presented. The denominators were the average of the population over ten-year period in each district.

Demographic characteristic

The demographic characteristics of *S. suis* cases in terms of population, sex and age were analyses by descriptive analysis as described below.

Population

The incidence rate of *S. suis* cases in Chiang Mai province were calculated for each year between 2005-2014. The incidence rates of *S. suis* cases for the rest of Thailand (excluding Chiang Mai) between 2009 to 2015 were also calculated. Incidence rates are given per 1,000,000 persons (number of reported cases divided by the population for that year multiplied by 1,000,000).

Age-specific cumulative incidence

The age-specific cumulative incidence of *S. suis* for Chiang Mai province was given per 10,000 persons (number of reported cases in each age group divided by the sum of the population over ten-year period multiplied by 10,000). Denominators were the sum of population at risk over ten-year period of each age group.

Cumulative incidence is a measure of frequency, a measure of disease frequency during a period of time, where the period of time considered is an entire lifetime (Reviews, 2015). Cumulative incidence is defined as the probability that an event, has occurred before a given time. The calculation uses all individuals in the population considered to be at risk for a disease over a specified time. It provides information of how far the disease has spread over that time. Cumulative incidence can be calculated as follows:

$$CI = \frac{Number\ of\ people\ who\ develop\ disease\ during\ the\ study\ period}{Number\ of\ people\ at\ risk\ of\ developing\ the\ disease\ at\ the\ start\ of\ the\ period}$$

Gender-specific ratio

Male and female *S. suis* cases occurring each year were calculated. The gender ratio (the proportion of males to females within a given population) is usually

expressed as the number of males per 100 females. The gender ratio was calculated for *S. suis* for the year where both male and female cases were reported. The gender ratio was calculated using the formula below:

$$Gender\ Ratio = \frac{\frac{Male\ case}{Male\ population}}{\frac{Female\ case}{Female\ population}}$$

2.4 Results

2.4.1 Annual human *S. suis* cases and deaths

Between 2005-2014 there were 257 confirmed human cases of *S. suis* across 20 of 25 districts of Chiang Mai province reported to Chiang Mai Health Office. Cases were reported every year and data from all cases were analysed (see Figure 2-6). The highest annual number of cases was 83 in 2008. Annual incidence rates varied from 1.21 to 49.69 per 1,000,000 populations. There were 26 case fatalities during this period. Case fatality by year varied from 1.20-100% (see Figure 2-7). The overall proportion of case fatalities was 10.12% (26/257). The outcome after treatment was reported for only 81 cases (31.5% of the total) of which 67.9% (55 cases) were fully recovered and 32.1% (26 cases) died.

Figure 2-6 Annual human *S. suis* incidence rate in Thailand per 1,000,000 populations by year.

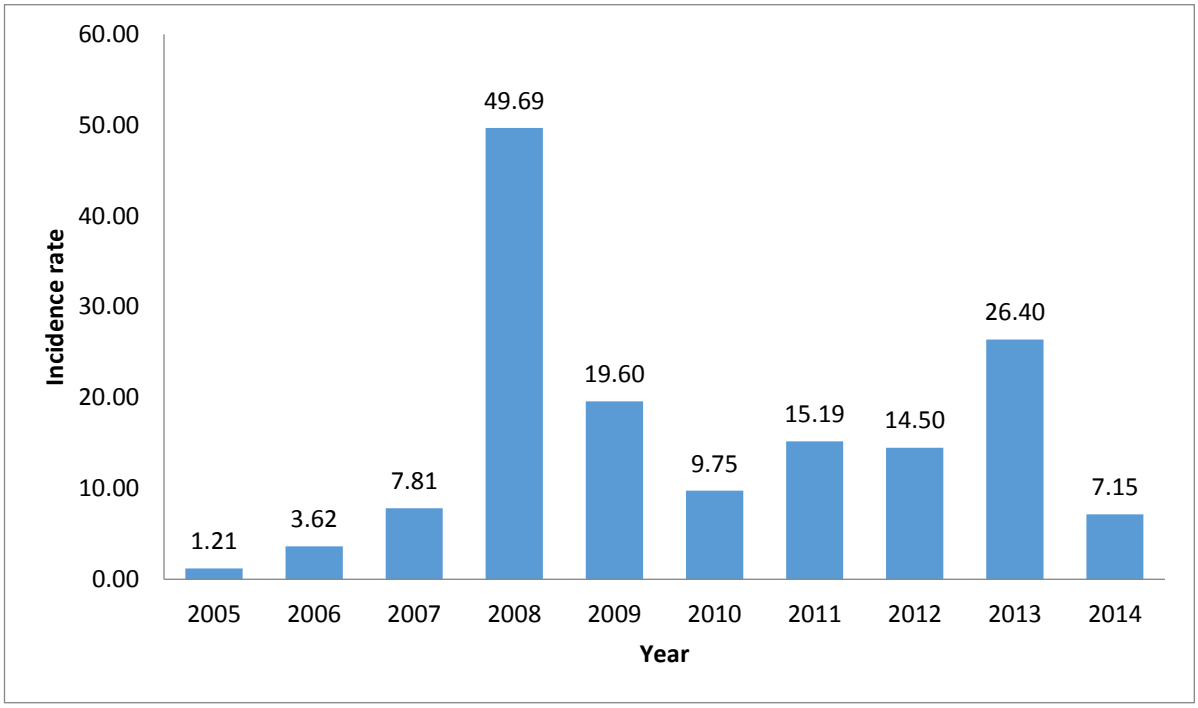
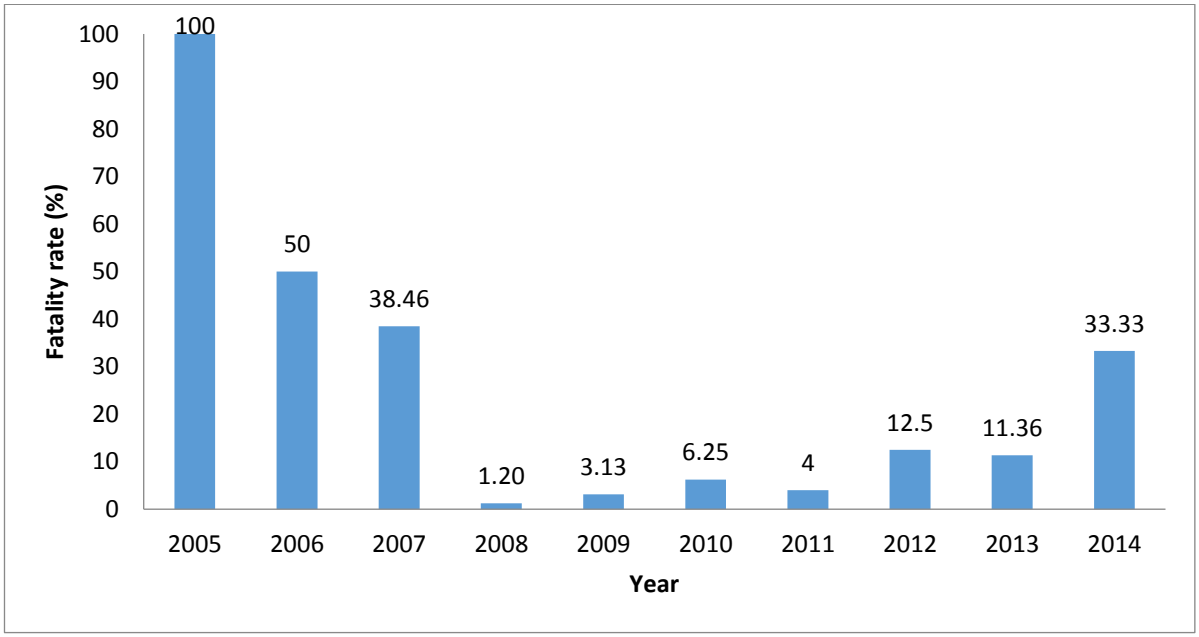


Figure 2-7 Fatality rate of human *S. suis* by year.



2.4.2 Monthly distribution of *S. suis* cases

Between 2005 to 2014, the highest case numbers occurred in July, followed by June and May, respectively (see Figure 2-8). Less cases occurred during the winter period from November to February. The general trend in monthly case numbers shows *S. suis* cases increasing, during the rainy period (May to October) and declining in winter (November to February). A peak in case numbers reported occurred in July in 2008, 2011 and 2012 (Figure 2-8Figure 2-9).

Figure 2-8 Total cases of *S. suis* by month over ten-year period from 2005-2014

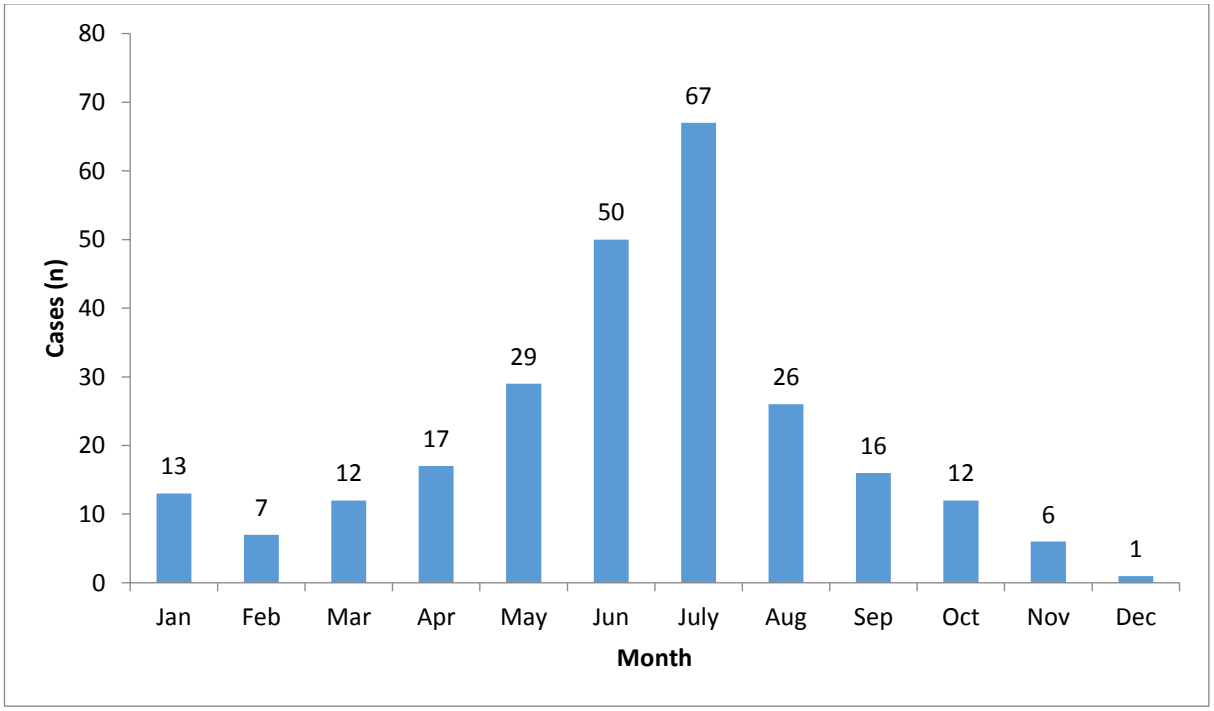
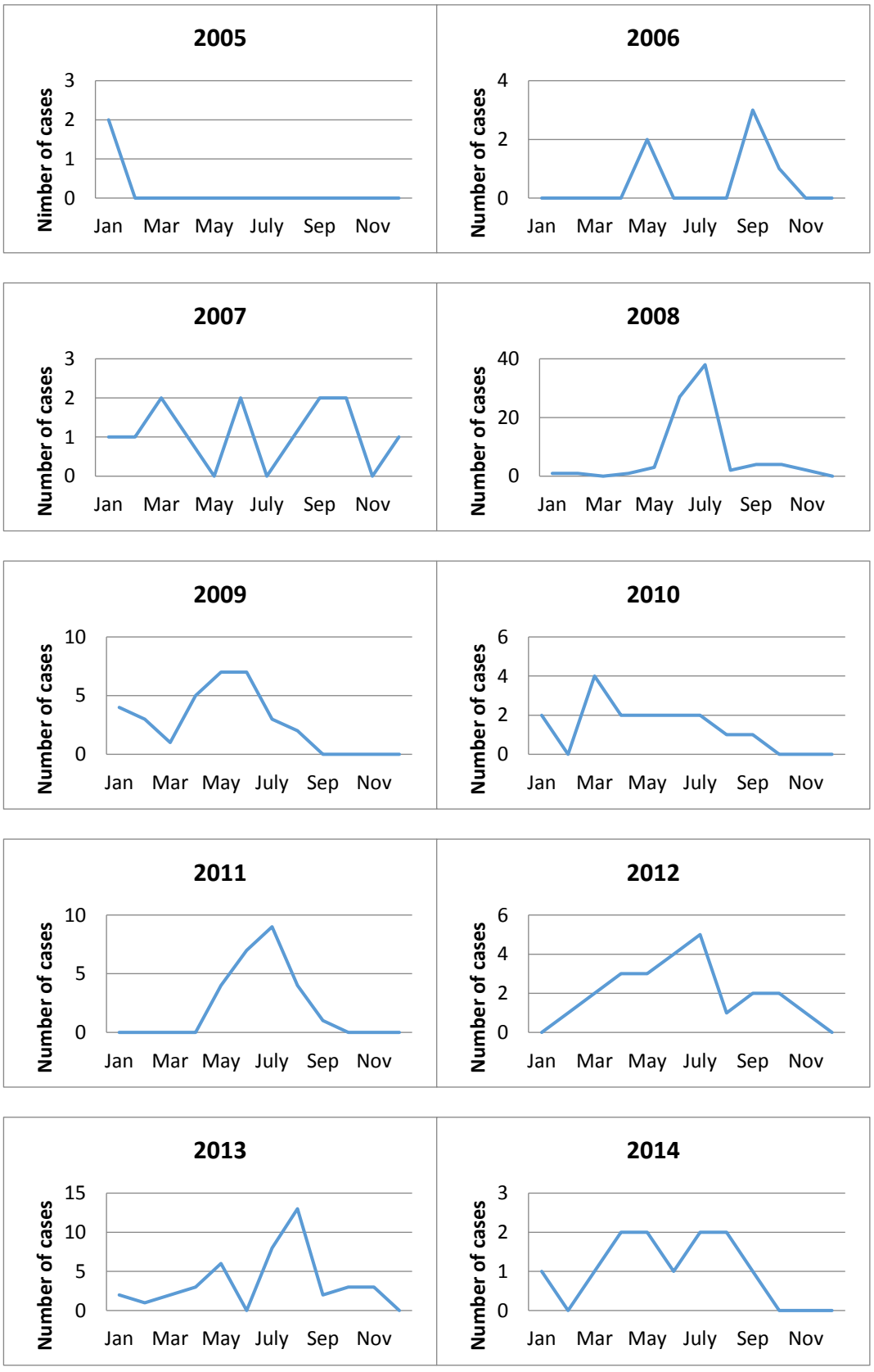


Figure 2-9 *S. suis* cases eah year by months



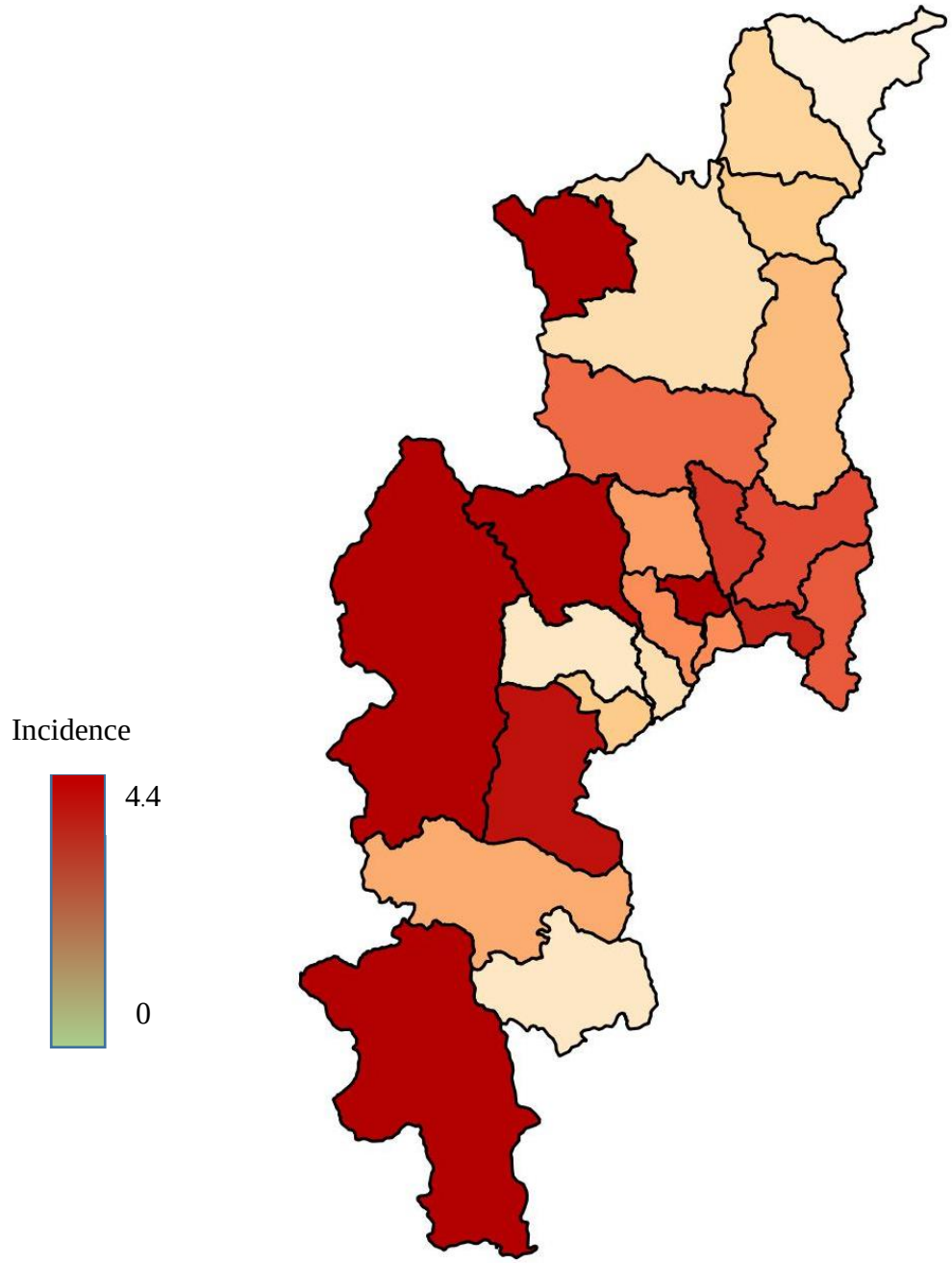
2.4.3 Distribution of human *S. suis*

Between 2005 to 2014 *S. suis* human cases were reported in 20 out of 25 districts in Chiang Mai. The distribution of *S. suis* cases within districts of Chiang Mai province are shown in Table 2-1**Error! Reference source not found.** and Figure 2-10. Most cases occurred in JomThong (n=78) followed by Doi Saket (n=45), SanKamPang (n=45) and Sansai (n=16). The total incidence rate for all districts was 17.12 per 100,000 people. The highest incidence was in JomThong district of 117.24 per 100,000, six times higher than for Chiang Mai province (17.12 per 100,000). No *S. suis* cases were reported from five districts, namely MaeJam, SaMeung, OmKoy, WiengHang and Kanlayaniwatthana.

Table 2-1 Incidence rates of human *S. suis* (per 100,000 person-year) from twenty reported districts in Chiang Mai between 2005-2014.

Districts	Population	Cases	Incidence rate
Muang	234,244	7	2.99
JomThong	66,531	78	117.24
ChiangDao	83,399	2	2.40
DoiSaket	70,215	45	64.09
MaeTang	75,044	9	11.99
MaeRim	88,835	9	10.13
Fang	112,847	6	5.32
MaeEye	73,537	2	2.72
Praw	49,324	3	6.08
SanPaTong	75,390	3	3.98
SanKampang	81,144	45	55.46
SanSai	127,062	16	12.59
HangDong	83,310	11	13.20
Hod	43,809	2	4.57
DoiTao	27,406	1	3.65
Saraphi	79,996	6	7.50
ChaiPrakarn	44,760	2	4.47
MaeWang	31,472	1	3.18
MaeOn	21,281	6	28.19
DoiLor	26,083	2	7.67
Total	**1,495,689**	**256**	**17.12**

Figure 2-10 Distribution of human *S. suis* in Chiang Mai



2.4.4 Demographic characteristic of *S. suis* cases in Chiang Mai

The incidence rate for *S. suis* in Chiang Mai between 2005 to 2014 was 1.21 to 49.69 per 1,000,000 population. The highest number of cases was observed in 2008 (83 cases) with an incidence rate of 49.69 per 1,000,000 population. The average incidence over the 10-year period was 15.52 per 1,000,000 population.

The number of reported *S. suis* cases from the rest of Thailand between 2009 and 2014 are shown in Table 2-2. The incidence rate varied from 1.70 to 3.37 per 1,000,000 population. The highest case numbers were reported in 2014 (214 cases) with the incidence rate of 3.37 per 1,000,000 population. The incidence rate of Chiang Mai people compared with the rest of Thailand showed that the Chiang Mai population had higher incidence rate 6.5 times than that for the rest of Thailand.

2.4.4.1 Age-specific *S. suis* cumulative incidence

The age-specific cumulative incidence of human *S. suis* in Chiang Mai and total numbers of cases in each age group over the 10-year period are shown in Table 2-3. The highest number of *S. suis* cases and the highest cumulative incidence was in the 51-60 age group (82 cases, CI=37.67% per 10 years per 10,000 people). The over 60 age group also presented with a high cumulative incidence for *S. suis* (CI=29.77% per ten-year per 10,000 people) and 41-50 age group (CI=26.48%). These three age groups had cumulative incidences higher than the overall cumulative incidence (overall CI= 16.69 per 10 years per 10,000 people).

Table 2-2 : Incidence of *S. suis* in Chiang Mai population and the rest of Thai population.

Year	Chiang Mai population	Chiang Mai Case	Incidence	Thailand-Chiang Mai population	Thailand case-Chiang Mai case	Incidence
2005	1,650,009	2	1.21			
2006	1,658,298	6	3.62			
2007	1,664,399	13	7.81			
2008	1,670,317	83	49.69			
2009	1,632,548	32	19.60	61,892,514	105	1.70
2010	1,640,479	16	9.75	62,237,788	169	2.72
2011	1,646,144	25	15.19	62,429,889	118	1.89
2012	1,655,642	24	14.50	62,801,053	155	2.47
2013	1,666,888	44	26.40	63,119,021	129	2.04
2014	1,678,284	12	7.15	63,446,432	214	3.37
Total	**16,563,008**	**257**	**15.52**	**375,926,697**	**890**	**2.37**

Table 2-3: Age-specific of human *S. suis* (per 10,000 person-years) over 10 years.

Age group	Cases	Population at risk over 10- year	CI% per ten-year per 10,000 populations
0-10	2	1848841	1.08
11-20	4	2170640	1.84
21-30	16	2382804	6.71
31-40	26	2268890	11.46
41-50	69	2605712	26.48
51-60	82	2177072	37.67
>60	58	1948349	29.77
Total	**257**	**15402308**	**16.69**

2.4.4.2 Gender distribution of *S. suis* cases

Of the 257 human *S. suis* cases, 210 were in males and 47 were in females. The number of male cases each year were higher than the number of female cases. In 2005, 2006 and 2011 cases were only reported in males. The overall male-to-female ratio varied from 2.1:1 to 10.1:1 (Table 2-4).

Table 2-4 Number of *S. suis* cases by sex and sex ratio

Year	Male case	Female case	Sex Ratio
2005	2	0	
2006	6	0	
2007	11	2	5.7:1
2008	66	17	4.0:1
2009	29	3	10.1:1
2010	13	3	4.5:1
2011	25	0	
2012	18	6	3.2:1
2013	32	12	2.8:1
2014	8	4	2.1:1

2.5 Discussion

Between 2005 and 2014, 257 *S. suis* cases and 26 deaths from *S. suis* occurred in Chiang Mai across 20 districts. There was one large outbreak in JomThong district

in 2008. The disease affected 20 districts. Most affected districts were in the central and southern parts of Chiang Mai province.

The number of *S. suis* cases reported from Chiang Mai province were lower in 2005 and 2006 than between 2007 to 2014. During 2005 and 2006, there were only 2 and 4 cases per year respectively. The incidence was low at around 1.21 and 3.62 per million populations, with high fatality of 100% and 50%, respectively. These figures may not reflect the true incidence or fatality rates for *S. suis* during that period. *S. suis* was only listed as a reportable disease in Chiang Mai in 2005, and there were only limited *S. suis* diagnostic tools available in the hospital laboratory (Donsakul, Dejthevaporn and Witoonpanich, 2003). *S. suis* was not routinely tested for and was often miss-classified as *S. viridans* and few *S. suis* cases were confirmed (Khadthasrima *et al.*, 2007a). A hospital study in northern Thailand, indicated that more than 70% of *S. suis* cases were misdiagnosed as *Streptococcus viridans* (Fongcom *et al.*, 2009). In addition, practitioners and public health officers were also unaware of *S. suis* as it was not considered to be an important inection at that time. The outbreak of *S. suis* in Phayao in 2007 resulted in increased awareness of *S. suis* (Khadthasrima *et al.*, 2007a) and thereafter, the incidence of *S. suis* doubled. It is unclear as to whether this is due to increased incidence or improved diagnostics.

By 2008, the incidence of *S. suis* was as high as 49.69 per million. This increase was largely due to the outbreak in JomThong district during June and July 2008, where 264 suspected *S. suis* cases arose, presenting with similar clinical symptoms of fever, headache, muscle pain, stiffness, hearing loss and where one death was reported. Blood tests and PCR confirmed *S. suis* serotype 2 in 33 patients (Jaturpahu, 2009). The fatality rate of this outbreak was surprisingly very low, this may have been because

the SRRT team rapidly investigated the outbreak and resulting in prompt treatment. The SRRT team also co-operated with provincial livestock officers to identify the source of *S. suis* and provided advice to the local community to eat only cooked meat. Farmers and butchers were advised not to handle pig carcasses with their bare hands. Testing for for *S. suis* in pigs and on chopping boards, disinfection of pig stalls and markets were also implemented (Jaturpahu, 2009).

Treatment outcomes from 31.5% of the *S. suis* cases were available at provincial level. Cases were reported when the patients were first diagnosed with *S. suis* (clinical symptoms and case history). Case reports were rarely updated, the surveillance protocol does not require an update of patient status unless they die. Most cases on the surveillance system were described as "under treatment".

Although there were no distinctive patterns for *S. suis* outbreaks in Chiang Mai, there were more *S. suis* cases reported between May and July, at the beginning of the rainy season in Thailand. Previous studies, have indicated that case reports for *S. suis* increased during the rainy season in northern Thailand, Vietnam and in Hong Kong (Kerdsin *et al.*, 2011a; Ma *et al.*, 2008; Wertheim *et al.*, 2009b). There are several possible reasons for an increase of *S. suis* cases in that period.

Firstly, pigs may become more infected during the rainy season as both temperature and humidity are more suitable for bacterial survival and increase the risk of *S. suis* infection and of co-infection with other micro-organsims, increasing susceptibility (Clifton-Hadley and Enright, 1984; Dee and Corey, 1993). Pigs are susceptible to stress caused by the high temperatures and stifling high humidity that usually occur prior to heavy rains, weakening their immune systems (Morrow-Tesch,

McGlone and Salak-Johnson, 1994). In addition, the incidence of mycotoxin contamination in feedstuffs in increased in the rainy season (Bintvihok and Davitiyananda, 2003; Wirodkul *et al.*, 2008) and various studies have shown that mycotoxins can cause immunosuppression (da Rocha *et al.*, 2014; Zain, 2011).

Secondly, from May through to July, there are many community social gatherings and festivals. These include Coronation day, Plantation day, and several Buddhist days, including the major festival of Buddhist lent in July. Buddhist lent lasts for a period of three months for the monks, who are not allowed to sleep outside their temple during this period. The next major festival is at the end of Buddhist lent in October. On Buddhist days, people normally bring food to offer to the monks and all leftover food will be shared as there is always more food to go around than people. People tend to arrange important ceremonies (weddings and house blessings ceremonys) before Buddhist lent, as the monks must attend and bless these events. Gatherings also occur during "Long Khaek", which involves the planting of rice which involves many farmers and some villagers. Traditional dishes are served at these events, and one indispensable dish for the northern region is "Laab". Laab, is a dish of minced meat mixed with dried spices and/or blood of the animal, which is of major socio-cultural importance in the northern region. Laab is considered a high-class dish for northern people, representing the economic and social class of people in northern society. Laab is a symbol of fortune and is a homonym to fortune in Thai language (Prompichai, 1999). Local peoples prefer to eat Laab raw (uncooked raw minced meat and blood) as they believe that it has more flavour than when it is cooked.

2.5.1 Distribution of human cases

Between 2005 and 2014, *S. suis* cases were reported from hospitals in 20 of 25 provinces in Chiang Mai. The districts with high incidence also had a high population density. These districts have large hospitals with laboratory facilities; or are near central district with good transportation and patient samples can be sent to provincial hospital laboratory or private laboratories. In small hospitals with limited laboratory facilities, patients are treated according to their clinical symptoms and *S. suis* cases are rarely reported unless there is a have history of pork consumption or they show specific symptoms, e.g. hearing loss.

Five districts of Chiang Mai reported no *S. suis* cases since 2005. People in these districts are from Luo, Pagagayor and Mon; minority ethnic groups that have no tradition of eating raw meat. These districts are small and are in remote difficult to access locations. The hospitals are also small and ill equipped to identify *S. suis*.

2.5.2 Demographic characteristics

Chiang Mai has a higher prevalence of *S. suis* than the rest of Thailand. Traditional raw meat dishes have a high impact in these northern communities. Laab and other traditional dishes are often eaten raw rather than cooked, and communities are unaware of the risks of unsanitary food preparation. In rural areas, some people cook unwashed meat as they believe water washes away the sweetness and nutrition of the meat. They also enjoy eating raw or semi-cooked pork (Kunnalok, 1996).

Chiang Mai province has the highest population in the north of around 1.6 million (Anon, 2015b). Pork consumption in Chiang Mai is 83-375 kilograms per

person per year, while the average consumption in Thailand is 13.0 kilogram per person per year (Pana-ananpaiboon, 2011). 100,000-500,000 pigs are slaughtered for meat in Chiang Mai each year. Chiang Mai has the highest number of pig holdings in Thailand (16,256), most of which are small holdings (12,208 units) and the highest number of native pigs at 84,587 head. There are 4,907 commercial pig holdings in Chiang Mai of 251,129 head (Department of Livestock Development, 2014).

Commercial pig farms in Thailand are advanced with conditions beyond the minimum standard required from the Department of Livestock Development. Most commercial farms are contracted to large companies and most pigs go to a standard slaughterhouse. In contrast the small or backyard farms may not meet the minimum standard practice for a pig farm. Sick pigs may be sold for slaughter or slaughtered locally (backyard) and distributed in the village (Rattanamaneekorn and Oopakornrat, 2014). The high consumption rate, the habit of eating raw or under cooked pork, and sub-standard pig holdings in Chiang Mai are major risk factors for *S. suis* infection.

Age

In this study, most *S. suis* cases were found in the 51-60 age group, followed by 60 years and over and then the 41-50 age group (CI=37.67%, 29.77% and 26.48% per ten years per 10,000 people, respectively). Previous studies show that *S. suis* are more likely to occur in patients from middle-aged to elderly. A hospital study in northern Thailand during 2010, found the average age of *S. suis* patients to be 52.02 years. The highest number of cases were found in the 60 years and over age group (35.0%), followed by 51-60 age group (20.0%), the 41-50 age group (18.3%) and the 31-40 age group (13.3%), respectively (Sittiroj, 2011). Other studies also have supported this age infection profile (Fongcom *et al.*, 2009; Kantu, Woradecha and

Niyompeng, 2008). The middle aged to elderly may be more vulnerable to infection since the immune system deteriorates with age (Miller, 1996) and underlying diseases (diabetes, high blood pressure, kidney, liver and heart disease) may increase susceptibility to infection (Fongcom *et al.*, 2009; Kantu *et al*., 2008; Sittiroj, 2011).

Sex

Sex is a major risk factor for *S. Suis* infection. In this study, 80% of *S. suis* cases were in males and males were 5.6 times more likely to be affected. Previous studies showed that 90% of *S. suis* infection was in males (Samerchea and Neungmek, 2008; Sittiroj, 2011) and that males were 3.1 times more likely to be cases (Fongcom *et al.*, 2009). Male behaviours may predispose them to *S. suis* infection, for example drinking alcohol (Fongcom *et al.*, 2001; Khadthasrima *et al.*, 2007a). Alcohol consumption may facilitate *S. suis* translocation across the intestines in humans who have liver disease (Nakayama *et al.*, 2013). A smoking and alcohol consumption survey in Thai citizens in 2014 showed that males drank 4.6 times more alcohol than females and that the frequency of drinking in males was 6 times higher than in females (National Statistical Office, 2015).

2.6 Limitations

S. suis is an underreported infection (Fongcom *et al.*, 2009). Underreporting and miss-classification are features common to all surveillance system (Gibbons *et al.*, 2014). Several issues influence under-reporting and underestimation of *S. suis* cases. Firstly, surveillance for *S. suis* is passive, data are subject to the ability of to capture and identify cases, which is dependent on the expertise of health practitioners, laboratory facilities and ability to refer for analysis. Secondly, there are limitations of

budgets for investigation and detection of cases. Thirdly, surveillance data are captured by the place of case residence, and do not reflect place of exposure, i.e. cases reported from different places may in fact come from the same source of origin. Thirdly, cases may not be recorded in the *S. suis* surveillance system, or may be entered without confirmation that the infection is indeed *S. suis.* The epidemiologist or health personnel in charge of reporting might fail to update the data in the surveillance system. Data quality may also be a problem. In many cases the data available from the surveillance system were incomplete. Missing data, e.g. the patient's status (under treatment, discharge from hospital, or follow up treatment), and patient outcome (fully recovered, ongoing treatment, disability) were lacking.

2.7 Summary

This retrospective study of human *S. suis* surveillance data in Chiang Mai between 2005-2014 showed that:

1) *S. suis* is endemic in Chiang Mai. Annual incidence rates varied from 1.21 to 49.69 per 1,000,000 population. The highest incidence rate was observed in 2008 at 49.69 per 1,000,000 population.

2) *S. suis* incidence rates in Chiang Mai were 6.5 times higher than the rest of Thailand (15.52 and 2.37 per 1,000,000 population, respectively).

3) *S. suis* cases were reported from 20 out of 25 districts in Chiang Mai. The overall incidence rate was 17.12 per 100,000 people. The greatest number of cases occurred in Jom Thongs (117.24 per 100,000 people).

4) No *S. suis* cases were reported from five districts of Chiang Mai (MaeJam, SaMeung, OmKoy, WiengHang, and Kanlayaniwattana). It is unknown as

to whether this is due to underreporting or differential exposure of these communities to *S. suis* risk factors.

5) Between 2005 and 2014 there was an observed trend for higher numbers of *S. suis* reported in May, June, July, and August.
6) The male to female ratio of *S. suis* infection was between 2.1:1 to 10.1:1. The number of male cases was higher than for females in each year.
7) *S. suis* had high incidence among individuals aged between 51-60 and in over 60-year age group (CI=37.67% and 29.77% per ten year per 10,000 people, respectively).
8) Overall case fatality for *S. suis* was 10.12% (26/257). Fatality rates peaked in 2007 (38.46%) and 2014 (33.33%).

CHAPTER 3-Burden of *Streptococcus suis*

3.1 Study aims

This study measured the burden of *S. suis* in Chiang Mai province in the northern region of Thailand using the Disability Adjusted Life Year (DALY) using *S. suis* surveillance data. The economic burden of *S. suis* to patients and the health system in Chiang Mai was also explored using *S. suis* patient experiences during illness, treatment and recovery in Chiang Mai province, focusing on psychological and economic impacts for the patient and their household, tracing the experience of the household from infection through to treatment pathways, livelihood impacts, treatment, recovery, death and disability.

3.2 Introduction

The Thai Ministry of Health, reported 496 *S. suis* cases between 2011 to 2013 with a mortality rate of 6.7% , and 75.6% and 24.4% morbidity in males and females respectively (Wongkamma *et al*., 2014). Outbreak investigations performed by the Surveillance Rapid and Response Team (SRRT), Ministry of Health, revealed that consumption of raw or semi-cooked meat and blood was the most significant risk factor for *S. suis* infection (Khadthasima *et al.*, 2007). *S. suis* infection can be fatal and individuals that survive may suffer from hearing loss.

To date there have been no studies of burden and economic impact on patients infected with *S. suis* at either national or local level in Thailand. The general population are not sufficiently aware of *S. suis* to avoid risky behaviors. Estimation of the disease burden and economic impact of *S. suis* infection is key for informed policy for *S. suis* surveillance and control.

The World Health Organization (WHO) and the World Bank developed the disability-adjusted life years (DALYs) method to estimate the overall disease burden in a population (Murray, 1994; The World Bank, 1993). The DALY is a single estimate that aggregates the burden of disease at a population level in global burden of disease studies (Murray *et al*., 1994; WHO, 2013).

DALYs do not provide a complete picture of the adverse impact of illness. Socio-economic impact measures are regularly used at societal and household level, for example, impact assessments on outbreaks affecting international trade or regarding treatment costs and losses in income to patient households (Conteh *et al*, 2010; WHO, 2009). Disease burden can be approached from the perspective of the individual patient, where infection has significant but slightly different on psychological and social impacts (Jones and Williams, 2004; Perera *et al.*, 2007). Disease impact is measured by the losses in manpower, income, and disability.

3.2.1 Burden of disease

Burden of disease refers to the impact of a health problem as measured by financial cost, mortality, morbidity, or other indicators. It is a method that combines data from multiple sources. The intention is to compare the fatal and non-fatal health loss from diseases or health states in the population (Holtz, 2012). Summary measures of population health (SMPH) are measures that combine information on mortality and non-fatal health outcomes. Various methods have been developed and the burden of diseases has been studied and published to calculate the impact of health problem (mortality, morbidity or disability) and can be divided into two categories; health expectancies and health gaps (Donev *et al.*, 2010).

- Health expectancies: is an estimate of the years in good health or bad health that a person can expect to live. The measures included in this group are: active life expectancy (ALE); disability-free life expectancy (DFLE); disability-adjusted life expectancy (DALE); healthy adjusted life expectancy (HALE) and quality adjusted life expectancy (QALE).

- Health gaps: is an estimate lost years of full health in comparison with some ideal health status or accepted standard (Donev *et al.*, 2010). The measures included in this group are: potential years of life lost (PYLL); healthy years of life lost (HYLL); quality adjusted life years (QALY) and disability adjusted life years (DALY).

3.2.2 Disability Adjusted Life Year *(*DALY*)*

The DALY was developed by Harvard School of Public Health in collaboration with the World Bank and WHO. Adopted by WHO in 1996 as a useful method to estimate the overall disease burden in a population, to gain evidence to enable health policy (WHO, 1996). The application of DALYs has "the potential to revolutionize the way in which we measure the impact of disease, how we choose interventions, and how we track the success or failure in our intervention" (Foege, 1994).

The first global burden of disease study was carried out by Murray and Lopez in 1990 (Murray *et al*., 1994). DALYs are the main measure employed by the World Bank and WHO for global burden of disease (GBD) estimations.

The DALY estimates overall disease burden and is presented as the number of the years lost due to health problem, disability, and early death. It is a time-based

measure that combines years of life lost due to premature mortality and years of life lost from living less than ideal health (Donev *et al.*, 2010). It is the sum of two components; the years of life lost (YLL) due to dying early, and years lived with disability (YLD) a component that measures the burden of living with a disability.

YLL refers to years lost due to premature mortality and is calculated by subtraction of the age of death from the standard life expectancy for a person. In the global burden study, the standardized maximum life span is taken as Japan, the country with highest life expectancy in the world (Donev *et al.*, 2010).

YLD are the years lived in a state of poor health or disability due to each disease and depends on the severity of the disability caused by that disease to the affected individual and the length of the disabled period. YLDs per person per sequeala are equal to the prevalence of the sequela multiplied by the disability weight for the health state associated with that sequela. YLDs for a disease or injury are the sum of the YLDs for each sequela associated with the disease or injury (Vos *et al.*, 2012).

The disability weight represents the severity of health loss associated with diseases and conditions in DALY estimation. In the initial GBD study in 1996, a large set of global disability weights was derived. In the 2010 GBD study, there were a number of revisions and an alternative set of disability weights was developed, covering 220 unique heath states (Salomon *et al.*, 2012). This was revised again for the 2013 GBD study as some disability weights in GBD 2010 were not represented and there was inconsistency of wording across different levels of severity for the same impairment. This included the disability weight of hearing loss, treated spinal cord

lesions and neck level. There are 235 unique health states with a disability weighting in the GBD 2013 study (Solomon *et al.*, 2015).

GBD studies have been criticised for their methodological and normative choices, including the use of age-weighing; the use of different life expectancies for men and women; discounting; and the determination of disabilities weights, which are intended to capture the severity of a condition. The 2010 GBD study team included experts in multiple disciplines to help revise the methodology. The DALY calculation was simplified, by making four changes including: dropped discounting; making age weights uniform; using the same life expectancy for men and women; and revising the methodology for determining disability weight (Voigt and King, 2014). The improvements added legitimacy but have not settled the debate over whether universal disability weights are possible, desirable or useful for policy (Voigt and King, 2014).

3.2.2.1 DALY in Thailand

Thailand uses DALYs to measure the disease burdens. Thailand has a data collection system that provides both mortality and morbidity data and has produced national level burden of disease assessments (Bundhamcharoen *et al.*, 2011). The first burden of disease study was undertaken in 1999, by the Burden of Disease Study Committee, Ministry of Public Health (Choprapawon *et al.*, 2005). Subsequent studies have been undertaken by the International Health Policy Program (IHPP) with support from the Thai Health Promotion Foundation (Bundhamcharoen *et al.*, 2011). According to the latest burden of disease study in Thailand in 2013 (International Health Policy Program, 2015), the total disease burden in the Thai population in 2011 amounts to 10.6 million DALY, 6.1 million in male and 4.5 million DALY in women. Males have a 1.4 times higher burden than females. The major contributers to the

disease burden in males were alcohol dependence/harm (8.8%), traffic accidents (8.0%) and stroke (6.9%). In females, the three major contributers to the disease burden, were stroke (8.2%), diabetes (7.8%) and depression (5.4%).

Fatal burdens (YLL) are the major component of the burden of disease. YLLs accounted for 3.9 million DALYs (57%) and 2.5 million DALYs (52%) of total DALY loss in men and women respectively. Traffic accidents were the leading cause of DALY and YLL in men and accounted for 12% of YLL, while stroke caused the highest YLL accounted of 11% in women. Stroke and diabetes were the second leading causes of YLL in men and women respectively. Other conditions in the top five ranking were liver cancer, ischemic heart disease and HIV/AIDS in men; ischemic heart disease, HIV/AIDs and traffic accidents in women.

Disability burden (YLD) amounted in total to 4.2 million YLD; 2.2 million YLD in men and 2 million YLD in women. Alcohol dependence/harm was the leading cause of YLD in men, responsible for 23% of total YLD. Other important conditions were cataract, depression, diabetes, and osteoarthritis. The leading YLD in women was depression, estimated at 11% of total YLD in women. Other major conditions contriburing to the ranking included cataracts, osteoarthritis, diabetes, and anaemia.

3.2.3 Public Health system of Thailand

Thailand has a population of around 65 million, with a decreasing growth rate of 0.8 percent per annum (Prasatkul and Wapattanawong, 2012). There is a proportional increase in the population of individuals of working age and older age categories. The population census shows change in age structure of the Thai population as Thailand is now entering the period of an ageing society with ageing index of

68.77% in 2013 (Ministry of Social Development and Human Security, 2013). The health status of the Thai people has been rapidly improving. Life expectancy at birth is estimated at 71.6 year for males and 78.4 year for females (National Statistic Office, no date). Thailand is facing problems with increasing incidence of non-communicable diseases, environmental health hazards and emerging infectious diseases. The Health Service over the last two decades has shifted to an integrated approach aligning prevention, promotion, curative and rehabilitative services (Tophothai *et al.*, 2013).

3.2.3.1 Health care delivery system in Thailand

The Ministry of Public Health is the main government body responsible for the oversight of Public Health in Thailand. Duties include oversight of national health policy and operating most government health facilities. The other non-ministerial government agencies include: the National Health Security Office (NHSO), responsible for allocating funding through the Universal Coverage Program; the Health System Research Institute (HSRI); the Thai Health Promotion Foundation (Thai Health); the National Health Commission Office (NHCO) and the Emergency Medical Institute of Thailand (EMIT).

The health service delivery system under Ministry of Public Health involves 9,755 health promotion hospitals, 787 district hospitals, 94 general hospitals, 89 specialized hospitals, and around 50,000 health care centres (Health Statistic Plan Sub-committee, 2013). Other government units and public organisations that operate hospitals are the military, universities, local governments and the Red Cross. Private hospital and clinics are regulated by the Medical Registration Division.

Public hospitals serve the local population and can be classified according to size and location. Sub-district health promotion hospitals are the primary healthcare units located in each sub-district and have duties on medical treatments, communicable disease control, and health promotion services. Health promotion hospitals are limited to providing primary care with no ability for patient to be admitted and most cases are referred to higher level hospitals. Community or district hospitals are located at the district level and can be classified by size, the large hospitals have a capacity of 90-150 beds, medium 60 beds, and small 10-30 beds. Community hospitals are usually limited on their ability to treat complex diseases, referring patients in need to more advance and specialized care to general and regional hospitals. General hospitals are situated in capitals of the province or in major districts and have a capacity of 200 to 500 beds. Lastly, regional hospitals located in province centre, have a capacity of at least 500 beds and have a comprehensive set of specialists on staff.

According to Chiang Mai Health Office, the public healthcare service in Chiang Mai comprised of 266 health promotion hospitals, 21 community hospitals, 1 general hospital, 1 university hospital, and 6 other public hospitals.

3.2.4 National Health Security System

Thailand is a middle-income country with an established national health security system for the entire population. Prior to 2002, health and medical provision for the Thai population was the responsibility of the Ministry of Public Health, but around 30% of the Thai population were without health coverage despite gradual extension of coverage to various population groups (Tangcharoensathien *et al.*, 2009). In 2002, Thailand established a universal health care system for Thai nationals - a tax-

based system. It comprised three main financial risk protection schemes: a civil servant medical benefit scheme for public employees and dependents; social health insurance for private employees, and the universal coverage scheme for the remaining population not covered by the former two schemes. Most people are under the universal coverage scheme (75.29%), followed by social health insurance (15.42%) and civil servant medical benefit scheme (7.89%). Under these health schemes, coverage of the health care in the populations reached 98% (Tangcharoensathien *et al.*, 2014).

The universal coverage scheme, previously the "30 baht treats all diseases project", is a public health insurance scheme that provides treatments to registered members for a co-payment of 30 baht (£0.60) per visit. This allows access to health services in the district, and if necessary, cases can be referred for specialist treatment. In 2006, the government abolished the 30-baht co-payment and made the universal coverage scheme free making healthcare is accessible to all Thai nationals and reducing the burden of health costs on the poor (Yiengprugsawan *et al.*, 2010).

Even though the universal coverage scheme made healthcare more accessible to middle and low income people in Thailand, problems persist. The high share of expenditure on the universal coverage scheme is shouldered by the government. Health expenditure across all health schemes is rising due to the increasing number of chronic diseases, an aging population, emerging diseases, etc. As a free-treatment system, people tend to seek care when they are sick rather than take care of their health. Health personnel have high workloads causing them to leave the public sector and that make the situation even worse. Budget and resources are distributed according to population size, but real patient numbers and disease complications make case management a challenge in some hospitals (Sakunphanit, n.d.).

The Thai health expenditure budget represented about 6.5% of the gross domestic product (GDP) in 2014, the second highest after Vietnam (see Table 3-1) (World Bank, 2016). Eighty per cent of health expenditure comes from the government. In 2015, the annual spending on health care amounted to 28,000 billion baht (£560 billion) (Bureau of the Budget, 2015). However, health expenditure increased around 7% per year while GDP increased only 5% per year (Anon, 2014c). The Thai demographic indicates an aging society and that by 2025 demands for health expenditure will increase by 3.6 times. (Anon, 2014c).

Table 3-1 Total health expenditure of the Southeast Asia countries in 2014

Countries	**Health expenditure (%GDP)**
Brunei	2.6
Burma	2.3
Cambodia	5.7
Indonesia	2.8
Laos	1.9
Malaysia	4.2
Philippines	4.7
Singapore	4.9
Thailand	6.5
Vietnam	7.1

3.2.5 Characteristic of the Thai economic system and society relevant to *S. suis*

The Thai economy was previously based on agriculture. When Thailand started the first National Economic and Social Development Plans in 1961, the Thai economy gradually shifted from agriculture to an industrial and service society. According to the Office of the National Economic and Social Development Board, between 2008 and 2012, the agriculture share was around 11.6-13.3% GDP and the industrial sector 86.7-88.4% GDP (The Office of SMEs Promotion, 2014). Despite agriculture having a small share for GDP, more than half of the Thai population earn their living from agriculture, mostly smallholders. The Agricultural Census in 2013, indicated 5.9 million agricultural holdings, an agricultural area of 1.8 million square kilometres (35.7% total land area). The average agricultural area per holding was around 31,000 square meters (National Statistic Office, 2013).

A labour force survey in May 2014 showed there were 37.75 million employed persons; 12.3 million in agriculture and 25.46 million in the non-agricultural sectors. When compared to the previous year, the number of employed persons in agriculture sector fell by 520 thousand, but increased in the non-agriculture sector by 80 thousand. Unemployment in Thailand was reported at 0.9% or 0.36 million persons, a large proportion working in substantial agriculture and vulnerable employment. The unemployment rate was increased from 2013 (National Statistics Office, 2014c).

Thailand is the second-largest economy in Southeast Asia, after Indonesia but ranks in the middle of Southeast Asia for GDP per capita, after Singapore, Brunei, and Malaysia. Exports accounting for more than two-thirds of the GDP (IBP, 2013).

According to the National Economic and Social Development Board, in 2014, Thailand had a GDP of 12,141 billion baht (£242.8 billion) (Ministry of Social Development and Human Security, 2015). After the Asian financial economic crisis in 1997 Thailand took ten years to regain its economic status (Moonchana, 2006) and Thailand encountered many indirect effects of the financial crisis. A coalition of protesters against the former prime minister resulted in military coups in 2006 and 2014 (Hewison, 2014; Pratumsawad, n.d.). In 2011 floods threatened the country for half year. The World Bank assessed the total damage and reported a cost of 1.425 trillion baht (£28.5 billion) (Kotsuki, Tanaka and Komori, n.d.).

3.2.5.1 Ethnicity

The Thai population is comprised of individuals from multiple ethnic backgrounds: 75% of the population is Thai ethnic; 14% Chinese; 3% Malaysian, the remainder being the various nationalities of hills tribes (National Statistic Office, 2014b). Chinese ancestry accounts for one third to one half of the Thai population. Most Isan people (originally from north-eastern region) are ethnic Lao intermixed with Khmer. There has been significant immigration from Myanmar, Vietnam, and Cambodia, including labourers for economic development in this sub-region (Soonthornthada, 2015). Thailand's population is mostly rural, according to the World Bank, in 2014, the rural population in Thailand was 50.83%, mostly concentrated in the agricultural area of the north-eastern, central and northern regions (World Bank, n.d.). It is difficult to quantify the number of people in urban areas. Millions of people migrate to Bangkok and other cities, returning to their place of origin, to work in the fields - officially resident in the rural area, but spending most time in the urban areas.

3.2.5.2 Religion

All Thai citizens have religious freedom. The main religion practiced is Buddhism of Theravada and religion plays an important role in Thai life and is an essential pillar of society. The religious demographic for 2000 showed 93.6% of the Thai population were Buddhists, 5.4% practiced Islam, 0.9% were Christian and 0.3% practiced other religious including Hindu, Chinese traditional religion (including Taoism) and the folk faiths of some ethnic groups (National Statistic Office, 2014b).

3.2.5.3 Education

Education in Thailand is mainly provided by the Thai government under the responsibility of the Ministry of Education. Free basic education for 12 years, from pre-school to high school, is guaranteed to all Thai citizens. A minimum of nine-year school attendance is mandatory. 96.7% of Thai population aged 15 years and older are literate (UNESCO, 2015). People living in urban areas spend more time in education higher than those in rural areas. In 2013, in the 15-59 years age group had average of 9.7 and 7.9 years in education in urban and rural areas, respectively (The National Economic and Social Development Plan, n.d.).

3.2.5.4 Food

In traditional Thai culture, rice is the first and most important part of any meal, normally served alongside other dishes. Various kinds of meats are served in every meal. In 2012, the consumption per capita of chicken, pork, and beef were 16.3, 14.85 and 2.28 kg, respectively (Thai Broiler Processing Exporters Association, n.d.; Beef Cattle Strategy Committee, 2013; The National Economic and Social Development Plan, 2012). Thai food varies across regions, people in different regions have their own

traditional dishes but food across all regions has the common feature of being spicy/hot especially in the southern and north-eastern regions.

Local dishes in some regions (especially the northern and north-eastern areas) are the source of pathogenic infections, include opisthorchiasis, trichinellosis and *S. suis*. Laab and lu, which comprise minced meat mixed with spices and/or blood, are the most common cause of *S. suis*. The Ministry of Public Health took measures to control *S. suis* in these regions, including campaigns to encourage people to eat cooked food in epidemic areas. However, many communities still favour eating raw meat, and this practice results in annual *S. suis* outbreaks. The ethnicity of *S. suis* cases is not recorded in the surveillance system, but can be assumed from the surname.

3.3 Methodology

3.3.1 Study method

The study of the burden of *S. suis* comprised two parts, firstly, the calculation of the DALY and secondly a calculation of the economic burden of *S. suis*.

3.3.2 *S. suis* burden*:* Disability Adjusted Life Year *(*DALY*)*

A quantitative assessment of *S. suis* was made by measuring DALYs in Chiang Mai Province population using 2013 as the reference year.

Data collection

S. suis is under surveillance in Thailand, hospitals are required to report *S. suis* infected patients to the Epidemiology section, Disease Prevention and Control. Data for the DALY study consisted of demographic baseline data (population, mortality) and disability incidence and duration data, disaggregated by age for the reference year.

Patient records were categorized by sex, age, nationality, address, outcome, onset date, admission date and discharge. Status and discharge dates were missing in some cases. To aggregate data, the hospitals that reported *S. suis* cases to the Chiang Mai Health Office were contacted to review the data on discharge date and outcome. Some cases needing further treatments, were not recorded in the patient charts. Patients were contacted by phone to get provide information on disability status after discharge.

Annual population data

Annual population demographic baseline data for Chiang Mai province were obtained from National Statistics office (www.nso.go.th) and the Ministry of the Interior (www.moi.go.th) (Table 3-2).

Rationale

The individual DALY was used in this study, since *S. suis* case numbers in 2013 were low. Individual DALY calculation values were considered as follows.

Table 3-2 Population of Chiang Mai province classsified by age and gender in 2013

Gender	Age group	Population
Male	0-14	128,526
	15-44	342,863
	45-64	217,426
	>65	71,978
Female	0-14	121230
	15-44	348200
	45-64	252928
	>65	85257
Total population		**1,318,652**

Disability weight (DW)

There is no disability weight for *S. suis* infection and so the disability weight for bacterial meningitis was used, as the disability weight due from many bacterial meningitis sequelae are the same. For the 2013 GBD study, disability weights were revised. Differing DW were related to differing health states, resulting in disability weights that were substantially different from GBD 2010 including for hearing loss (e.g. severe hearing loss with ringing : GBD 2010 0.032 (0.018-0.051); GBD 2013 0.261 (0.175-0.360) (Solomon *et al.*, 2015; WHO, 2013). In the GBD 2013 study, disability weights for 235 unique health states were revised. The disability weight used for YLD calculation for a patient with hearing loss was 0.239. For patients with *S. suis* infection without sequelae, the average disability weight from infectious disease severe and post-acute effects of 0.176 was used (Solomon *et al.*, 2015).

Life expectancy

A life expectancy value of 74 years for men and women, was used to calculate DALYs, as used in the 2010 GBD study.

Discounting and age weighting

Discounting rates and age weighting were not used in this study. These were dropped in the 2010 GBD study.

DALY calculation using individual based approach

DALYs for age-sex group are calculated as the sum of the non-fatal burden (YLD) and the burden of premature mortality (YLL):

$$DALY = YLD + YLL$$

From the formulas to calculate individual DALYs as presented by Zhang et al., 2010, YLL and YLD were calculated as follows.

Year of Life Lost (YLL) is used to measure the burden of premature mortality. This uses the expectation of life based in some ideal standard to estimate the loss of years of life associated with death. The life expectancy observed for Thai nation of 86 years was used (National Statistic Office, 2016). YLL is equal to *L* when *L* was the standard life expectancy at age of death in years.

$$YLL = L$$

Year of Life Lost due to Disability (YLD) are the disability component of DALYs. The basic formula for calculating individual YLD is as follows where *DW* is the disability weight and *L* is the average duration of disability.

$$YLD = DW \times L$$

3.3.3 *S. suis* burden*:* Economic Burden

Ethical approval

This study was coordinated and approved by Chiang Mai provincial Health Office and Veterinary Research and Development Center (Upper northern region), Thailand. Ethical consent was obtained from Chiang Mai Provincial Health Office.

Study design

This study design applied a cross-sectional survey of the economic burden in *S. suis* in Chiang Mai, Thailand during 2013-2014. Qualitative and quantitative interviews were applied to patients and healthcare personal between 2013-2014; 68

diagnosed *S. suis* cases that reported to Chiang Mai Provincial Health Office between 2013-2014 were used in this study.

Study method

To assess the economic burden of the *S. suis* patient, the interview and questionnaire survey studies were applied. The cost due to *S. suis* consisted of two parts; out of pocket payments (direct and indirect), and expenditure covered by the national health security. Patients and hospital personnel were interviewed and were contacted in advance to request permission for a face to face, or phone interview.

Patient interviews

Interviews were undertaken with *S. suis* patients diagnosed between 2013 and 2014. A total of 68 diagnosed *S. suis* patients, reported between January 2013-December 2014 were targeted for this study. Patients were contacted in advance to ask for permission for interview either by phone or face-to-face. Twenty-two patients participated in this study giving a response rate of 39.3%. The interview involved a range of open-ended and close-ended questions including:

i) Socio-demographic characteristics: patient characteristics, education, household role, income source, knowledge of *S. suis*, risk behavior etc;

ii) Health seeking behavior: date of onset of illness, symptoms, treatment duration etc.

iii) Burden of illness: disability, suffering from disability, treatment and follow up costs, time to full recovery, household affected etc.

To assess the socio-demographic characteristics, age, gender, education level, occupation and household income were surveyed and classified. Age was recorded as actual age when sick and reclassified into age groups (0-14, 15-44, 45-64 and 65+). Education levels were categorized into: no education; primary level; secondary level and college/university level or above. Household income was classified by the average monthly household income into low (0-9,250 baht (£0-185)), mid-low (9,251-11,000 bath (£185-220)), mid-high (11,001-25,000 baht (£220-500)) and high (more than 25,000 (more than £500)), based on quartile of mean household income in this survey. Health status was assessed by an interview with structured questionnaire.

Healthcare personnel questionnaire

Healthcare personnel in the hospitals were asked to complete a healthcare personnel questionnaire. From the *S. suis* surveillance record, all patients were treated at secondary (district hospitals), and tertiary care (provincial hospitals). Real treatment costs were available from these hospitals. Six hospitals reported *S. suis* cases at their hospitals during 2013-2014 and data was collected from all these hospitals.

The healthcare personnel were asked to provide information about i) Health center characteristics: size of hospital, coverage service area, coverage service population, number and role of health care personnel, average patient per day, diagnosis facilities. ii) Health care costs: treatment, drug used, referral of patient, cost of treatment, and other cost such as hearing aid and cochlear implantation.

Data analysis

Interview data were entered and analysed using Excel (Microsoft Office Excel 2013). Qualitative data were entered in Microsoft Word and analysed manually based on coding and memo writing.

3.4 Results

3.4.1 Health Burden

In 2013, in total 44 records of *S. suis* were recorded in Chiang Mai province of which there were 4 were deaths and 40 were reported cases. The incidence rate was 26.4 per 1,000,000 population, with a fatality rate of 11.36%. The mean age of acute *S. suis* infection was 55 years and the mean age of death was 60.5 years. A summary of the health burden is shown in Table 3-3.

3.4.1.1 Year of Life Lost (YLL)

Among the *S. suis* cases, 65 YLL were estimated, which generated a rate of 3.71 per 100,000 populations. The burden due to life lost from *S. suis* in males and females were 49 and 16 YLLs, respectively (5.74 per 100,000 male population, and 2.00 per 100,000 female population. Most *S. suis* YLL was due to males. The burden of *S. suis* in males presented in YLL in the age group from 15-44 and 45-64 years amounted to 8.56 and 6.95 per 100,000 populations respectively, while YLL in the 60 years plus age group was not calculated as the age of patients at death was more than the life expectancy of Thai people. There was only one female death case presented in the 45-64 age group, represented YLL of 5.87 per 100,000 population.

3.4.1.2 Year of Live with disability (YLD)

YLD were estimated at 3.7 per 100,000 population. The burden of hearing and deafness presented the biggest proportion of 99.6%, while the burden of acute *S. suis* infection contributed a small amount at 0.4%. The YLD majority was due to males, which generated 6.0 per 100,000 male population, while females amounted to 1.5 per 100,000. The burden presented as YLD was high in age group from 45-64 years and accounted for 5.16 per 100,000 population, followed by age group of 15-44 years and 65 years and over at 4.70 and 1.10 per 100,000 population, respectively.

3.4.1.3 Disability Adjusted Life Year (DALY)

From the population perspective, the total DALYs amounted to 129.81 or 7.41 per 100,000 population, while from the individual perspective they amounted to 2.95 per case. The disease was slightly dominated by the burden of premature death due to *S. suis* (50.0%), followed by hearing loss or deafness sequelae (49.8%), while the burden due to the acute disease of *S. suis* reflects the smallest proportion (0.2%). The burden of *S. suis* expressed in DALY in the age group from 15-44 years amounted to 52.3% of the total burden, whereas, the age group from 45-64 years amounted to 46.0%. The burden to males amounted to 11.72 DALY per 100,000 population, 3.5 times higher than females with DALY amounting to 3.30 per 100,000 population.

Table 3-3 Berden of *S. suis* in Chiang Mai in 2013

Health outcome	Case	YLLs	YLDs	DALYs	DALY per case	DALY per 100,000 populations
Acute *S. suis* disease						
Male						
15-44	3	0	0.04	0.04	0.001	0.010
45-64	12	0	0.09	0.09	0.003	0.036
65 and over	7	0	0.07	0.07	0.002	0.070
Female						
15-44	2	0	0.01	0.01	0.001	0.004
45-64	5	0	0.03	0.03	0.003	0.012
65 and over	1	0	0.01	0.01	0.001	0.014
Hearing loss or deafness sequelae						
Male						
15-44	4	0	35.85	35.85	1.12	9.59
45-64	3	0	15.08	15.08	0.47	6.17
65 and over	0	0	0	0.00	0	0
Female						
15-44	0	0	0	0.00	0	0
45-64	2	0	11.47	11.47	0.96	4.21
65 and over	1	0	2.15	2.15	0.18	2.01
Total *S. suis* infection and sequelae	**40**	**0**	**64.81**	**64.81**	**1.47**	**3.70**
Death due to *S. suis*						
Male						
15-44	1	32	0	32	1.00	8.56
45-64	1	17	0	17	0.53	6.95
65 and over	1	0	0	0	0.00	0.00
Female						
15-44	0	0	0	0	0	0
45-64	1	16	0	16	1.33	5.87
65 and over	0	0	0	0	0	0
Total death due to *S. suis*	**4**	**65**	**0**	**65**	**1.48**	**3.71**
Total *S. suis* case in male	**32**	**49**	**51.12**	**100.12**	**3.13**	**11.72**
Total *S. suis* case in female	**12**	**16**	**13.68**	**29.68**	**2.47**	**3.30**
All health outcome	**44**	**65**	**64.81**	**129.81**	**2.95**	**7.41**

3.4.2 Estimation of economic losses

3.4.2.1 Cost and impact of *S. suis* to patients and their households

In total 22 interviewees, former *S. suis* patients or their families participated in this study in Chiang Mai between 2013 to 2014.

Patient characteristics

Of the 22 patients; 12 were in the 45-64 year age group, 7 in age group 16-44 and 3 in age group 65 years and over (3). Households consisted of 1-4 members (81.8%) and more than 4 members (18.2%). The role of the patient in the household were household head (63.6%) or tenant, spouse, son or daughter of the household head (36.36%). The education level for S. *suis* cases was low, 50% spent four years or less in education (lower than primary level), and others were graduated from secondary level (36.36%) and tertiary level (13.64%). Other than two patients who were retired with a government pension, other main income generation activities included being a trader (36.36%), daily hired labourers (22.73%), agricultural sector (18.18%), and paid employment (13.64%). The 22 households reported to earn an average monthly income at 17,123 baht (£342), with 8 households above this average and 14 below it.

Knowledge of disease

Most of the individuals interviewed knew that eating raw or uncooked pork, or eating raw traditional dishes could cause foodborne disease. Only half of those interviewed were aware of *S. suis* disease before getting infected. Most had never seen any risk communication materials such as poster or stickers from Public Health organisations. Most patients (72.7%, 95%CI=51.9-86.8) realized that they should cook food thoroughly before eating it but more than 95% still preferred eating raw

traditional dishes, that they claimed were more delicious raw than cooked. Most patients (72.2%) drank alcohol; of these 56.3% were drinking daily or every other day; 12.5% at least once a week and 31.25% took alcohol infrequently.

Illness and treatment seeking behavior

Around 60% of the patients sought medical care within one day of experiencing clinical signs, with a maximum period of 4 days. Eighty-two per cent of patients went straight to hospital or to another health provider (private clinic or healthcare centre) and 18% had self-medicated prior to visiting a hospital. The most common clinical sign in *S. suis* patients were headache, fever, muscle pain, loss of balance, diarrhoea, and hearing loss. Other minor signs included stiff neck, seizure, shock, arthritis, blurred vision and vomiting. Although the average distance to the nearest hospitals was 5.3 km from their residence, the average distance from the patient residence to the hospital where they received treatment was 15.5 km. Hospitals in these communities were primary health care units that were not able to manage *S. suis* patients. Fourteen patients visited hospitals using their own car or in the car of a relative, four travelled by motorbike, the other four by cars of other people (rescuers car or ambulance).

Expenditures before and during treatment paid by patients

Most patients went straight to hospital, where they were covered by one of the national health security schemes. Some patients (27.27%) visited private clinics and private hospitals, not covered by any schemes. The average out of pocket for patients was estimated at 5,198 baht (£104). Most patients spent up to 500 baht (£10), while one case spent 2,000 baht (£40) at private clinics. One patient paid 60,000 baht (£1,200) for treatment at a private hospital before being transferred to a public hospital. Although all patients did not pay any for their treatment at the hospitals, a variety of

other expenses presented themselves. This included: special equipment, drugs or special tests costing around 500 baht and over; transportation to and from hospital for the patients and their relatives, and expenditure for relatives caring for the patients (meals, transportation etc.). Most expenditure was paid by the relatives who accompanied the patients at the hospitals. At least one relative stayed with the patient at the hospital, or stayed during the daytime if patients were admitted to the hospital care unit, to care for the patients. Some patients (36.36%) had more than one family member staying with them. The average transportation cost for patients to visit the hospital cost 148 baht (£3); most patients (31.8%) spent 200 baht (£40) for transportation. The expenditure for relatives who took care of the patients at the hospital (food and transportation) ranged between 100-720 baht per day, with an average of 293 baht per day. The average total expenditure for the person who accompanied the patient during their treatment was estimated at 5,050 baht (minimum of 300 baht and maximum 17,400 baht) depending on the duration of hospitalisation. The period of admission ranged between 2-87 days. Between 10-30 days for 50% of the patients; the other 41% stayed less than 10 days and 9% stay longer than 30 days.

The 22 patients were all covered by one of the national health insurance schemes. The majority were the universal coverage scheme (54.6%, 95%CI=34.7-73.1), social insurance (31.8%, 95%CI=16.4-52.7), and the civil servant medical benefit scheme (13.6%, 95%CI=4.8-33.3). Out of all the patients, 17 did not pay anything to the hospital, other payments were made for a private room, special tests, and special medicine and equipment. While most patients could meet these costs; six patients had to borrow money (range 10,000-50,000 baht; £200-1,000).

Apart from the two patients that were retired, only five patients needed some person to cover their work. Two hired labor for 15 and 45 days, while the other three were replaced by another person in their household. The hire rate was around 300 baht (£6) per day. Thirteen of the patients interviewed had follow up treatment after discharge from hospitals including physical therapy and disability treatment. Six had a follow up period of more than four weeks, five for 1-2 weeks, and two for 3-4 weeks. One patient paid 10,000 baht (£200) for a follow up special examination.

Impact to households and patients

Patients were asked about their health situation. Eleven had fully recovered, two had died, six had hearing loss or deafness, and two had generalized weakness and one with adhesive joints. The biggest household impacts were psychological (40.91%) and economic impact (36.36%). The biggest concern was that the family feared that the patients might not recover or die. For households reporting economic impacts, there were two main issues, firstly, that the patients were head of the households and were responsible for the main income, and secondly those families that had borrowed money during illness. Fifty-nine per cent of the patients experienced impact from disability, depression and resignation.

3.4.2.2 Cost of *S. suis* treatment through the national health security schemes

To derive the cost of *S. suis* treatment, the treatment costs at the hospitals were determined. Six healthcare personnel from hospitals that had reported *S. suis* cases to the surveillance system during the study period were interviewed.

Characteristic of the hospitals

Five district hospitals and one provincial hospital reported *S. suis* cases during 2013-2014. Three were small size district hospitals (around 30 beds), one was a medium sized district hospital (60 beds), and one was large (200 beds). The provincial hospital had a bed capacity of 800. The staff in small district hospitals included three to five general practitioners (GPs), none to two specialists in any area, less than 50 nurses, 10-45 assistant nurses, and one to two epidemiologists. The average number of patients were around 100 per day. In the medium size district hospital, there were ten GPs, two specialists, 100 nurses, and two epidemiologists, with average patients of around 200 per day. The large size hospitals had more than ten GPs, 28 specialists, 150 nurses, and three epidemiologists, with average patients of around 300 per day. The provincial hospital had around 10 GPs, 100 specialists, 450 nurses, and three epidemiologists, with the average patients more than 1,000 per day.

Laboratory facilities

Most district hospitals were not able to make a laboratory diagnosis and sent samples to either provincial or private laboratories. One hospital diagnosed *S. suis* based on primary bacterial culture results, with clinical signs and history taking. A primary diagnosis was made in 1-2 days, with up to 14 days for confirmation.

Hospital referred

Three small and one medium size hospitals referred *S. suis* patients to a provincial hospital, referral depending on the severity of their condition. The average transfer cost of 940 baht (£19), was covered by the departure hospital, ranging from 500-2,000 baht (£10-40) according to distance between the two hospitals and the number of medical personnel needed to travel with the patient.

Treatment costs

The large district hospitals provided exact treatment costs of *S. suis* from 2008-2013 (Table 3-4). Records were provided for 2008, 2010, 2011 and 2013 when the hospitals had *S. suis* patients (38 *S. suis* patients in 2008, when there was a large outbreak and three cases in each of the three other years. Expenditure was divided into medicine, laboratory costs, and other cost (treatment fee, room etc.). For 2013, the average treatment was 19,711 baht (£394) per patient; average medicine cost 2,724 baht (£54.50), laboratory 2,776 baht (£55.50), other costs 12,370 baht (£247).

Costs contributing to the economic burden of *S. suis* are shown in **Error! Reference source not found.**. Hospital costs are paid by the government. The average treatment cost was 33,455 baht (£669), varying according to case severity (1,000 baht (£20) to 145,000 baht (£2,900)). High treatment costs were due to secondary infections and other diseases. Average follow up costs were 4,500 baht (1,500-7,500 baht) or £90 (£300-250)).

Where hospitals undertook disease investigations, the cost of investigation ranged between 3,000-10,000 baht (£60-200), depending on the number of persons in the investigation team. If the patients suffered from hearing loss, a hearing aid costs around 7,000-30,000 baht (£140-600). For deafness that can be treated by hearing implantation, the cost is around 1,000,000 baht (£20,000) per year.

The cost of *S. suis* in 2013, for all 42 patients in Chiang Mai was 1,812,434 baht (£36,429) and for the whole of Thailand in 2013 was 8,155,954 baht (£163,199).

For 22 patients, the out of pocket expenditure was estimated at 5,198 baht (£104) per person. National security paid 37,955 baht (£759) per person rising to up to 43,153 baht (£863) per patient.

Table 3-4 Average *S. suis* treatment cost (baht and pound) per person at a hospital in Chiang Mai

Year	Patients	Medicine cost (range) Baht	Medicine cost (range) Pound	Laboratory cost (range) Baht	Laboratory cost (range) Pound	Others (range) Baht	Others (range) Pound	Total Baht	Total Pound
2013	3	2,724 (1,051-3,071)	54.5 (201-61.5)	2,776 (1,880-3,505)	55.5 (37.6-70)	12,370 (16411-22,926)	247.4 (328.2-458.5)	19,711	394.2
2011	3	3,300 (2,192-4,635)	66 (43.8-92.7)	2,810 (2,245-3,350)	56.2 (45-67)	10,063 (5,639-13,760)	201.3 (112.8-275.2)	16,569	331.4
2010	4	4,435 (1,348-9,223)	88.7 (27-184.5)	1,797 (1,320-2,400)	36 (26.4-48)	5,927 (2,300-9,700)	118.5 (46-194)	17,303	346.1
2008	34	8,724 (201-44,608)	174.5 (4-896)	2,432 (320-19,620)	48.6 (7.2-392.4)	18,132 (1,160-40,902)	362.6 (23.2-818)	31,848	637.0

Table 3-5 : Economic burden of *S. suis* in Chiang Mai

Title	Cost/person (Baht)	Range (Baht)	Mode/N	Mean	Cost (interview person n=22)	Estimate cost/year for patient in Chiang Mai (n=42)	Estimate cost /year for patient in Thailand (n=189)
Patient's out-of-pocket payment							
Cost before visit hospital		0-2000	0, 17				
Cost of travel to hospital		50-200	200, 7	148.2			
Cost of accompanied person (transportation and food) per day		100-720		293.2			
Cost of accompanied person (transportation and food) per day		300-17400		5,050			
Other extra cost (private room, extra test, extra medicine etc.)		0-60,000	0, 17				
Expenditure responsible by national health insurance scheme							
Treatment cost		1,000-145,812		33,455			
Refer cost (vehicle and medical personnel) (5 hospitals)		500-2,000		940			
Follow up cost (2 hospitals)		1,500-7,500		4,500			
Disease investigation cost		3,000-10,000					
Total expenditures							
Source of payment							
Out of pocket**	5,198				114,360	218,324.40	982,459.80
Universal health insurance***	37,955				835,010	1,594,110.00	7,173,495.00
SUM	**43,153**				**949,370**	**1,812,434.40**	**8,155,954.80**
£ (50 baht/£)	**863.06**				**18,987.41**	**36,248.69**	**163,119.10**

**exclude cost before visit hospital, follow up cost, treatment cost at private hospital, and other extra paid at hospital

***exclude refer cost

3.5 Discussion

The health impact of *S. suis* in Chiang Mai in 2013 and the economic impact of *S. suis* in 2013-2014 have been estimated. This is the first study to estimate the burden of *S. suis* in terms of DALYs. For *S. suis* disease in Chiang Mai in 2013, the total DALYs amounted to 129.81 (7.41 per 100,000 population), while from the individual perspective they were 2.95 DALYs per case. Most of the burden was in adults between 15-64 years (98.28%). No burden was present in those below 15 years, and the burden in the edler age group of over 60 was low at 1.72%. Hearing loss and deafness sequelae had significant impacts on patients' quality of life.

S. suis data from disease surveillance, hospital records and interviews in Chiang Mai were used for the DALY calculation but disability weights for *S. suis*, and outcomes after hospitalisation were not available. Of 44 cases, there were 19 cases (43.18%) that were assumed fully recovered and the real burden of *S. suis* may be higher than estimated, if these patients experienced sequelae.

The infectious diseases burden in Thailand in 2011 amounted to 761,009 DALYs, which was 480,006 and 281,004 DALYs or 1132.45 and 714.29 per 100,000 population in males and females, respectively (International Health Policy Program, 2011a). *S. suis* in Chiang Mai reflects 0.027% of the country's infectious disease burden. The total health burden of Thai people in the northern region was 77 per 100,000 population, while the *S. suis* burden from this study showed 7.41 per 100,000 population (International Health Policy Program, 2011b). Males had higher burden than females, which correspond to the burden of infectious diseases in national level.

While measuring morbidity and mortality is key for estimating the burden of disease in populations, an analysis of the economic impact of disease can provide policy makers with information down to microeconomic level of households. Of the 56 patients in the *S. suis* surveillance system of Chiang Mai Health Office between 2013 and 2014, 22 patients or close relatives to a deceased patient were available for interview. The remaining patients could not be contacted. The total expenditure for a *S. suis* patient was estimated at 43,153 baht (£863.06), 5,198 baht (£103.96) in out of pocket expenditure for the patient and 37,955 (£759.10) to the National Health Insurance scheme. Most of the expenditure for the patient were the costs incurred by the relatives of the patient, this included transportation and food, and was on average 293 baht (£5.86) per day or 4,820 baht (£96.4) over the period of hospitalisation. In Thai culture, it is common to have someone accompany the patient at the hospital. If patients stay in private room then a relative can stay with the patient in the room. If the patient stays in on a ward room the relative will come to care for the patient during the day. As most of the relatives reported living close to the hospital and they would visit each day bringing food or buying food at the hospital. The average monthly income of the households in this study was 17,123 baht (£342.46), the out of pocket expenditure incurred caring for an *S. suis* patient was equivalent to 30.36% of the average household monthly income. However, in this study most household incomes fell below this average and out of pocket payments were as high as 108% for the household with the lowest income (less than 5,000 baht per month).

S. suis cases were usually reported from secondary and tertiary medical centres since most *S. suis* patients had severe acute onset of disease and needed intensive treatment. Although patients visited the primary health facility first, they were referred

to a higher level of medical care. All expenditure associated with treatment (admission, laboratory tests, transportation for referral cases) were the responsibility of the government through the National Security Scheme.

Thai health expenditure is more than 20 billion baht (£400 million) each year. The majority of Thai citizen are in the universal health coverage scheme, the payment rate for one beneficiary registered under this scheme was 2,895.09 baht (£57.9) in 2015 and 3,028.94 baht (£60.58) in 2016 (National Health Security Office, 2015). The average *S. suis* treatment cost was 43,153 baht or £863.06 (ranging from 1,000 to 140,000 baht or £200 - £2,800). Follow up treatment costs added between 1,500 - 7,500 baht to these costs. The average cost for *S. suis* treatment was 11.56 (0.34 - 48.36) times higher than universal coverage for each registered beneficiary.

There is no surveillance of monitoring for *S. suis* in farms and slaughterhouses in Thailand and there is no vaccine available that is effective. Available vaccines are based on virulent proteins (eg. suilysin, muraminidase) (Du *et al.*, 2013), capsule polysaccharides, and surface antigen 1 (Hsueh *et al.*, 2014). Vaccines based on capsule polysaccharides give poor immunogenicity. Vaccines based on surface antigen 1 and bacterin provide poor immunogenicity and incomplete protection. Many strains that are virulent in pigs do not express these proteins. Some provide protection against infection only a few strains, and do not provide immunity to all virulent strains (Chen *et al.*, 2010; Baums *et al.*, 2009; Jiang *et al.*, 2016). If protection were only needed against *S. suis* serotype 2, which has the highest virulence in both pigs and humans, then it may be worth investing in vaccination (Goyette-Desjardins *et al.*, 2014).

Although, *S. suis* is an important zoonoses from a public health perspective, the low prevalence does not justify investment in monitoring and surveillance. *S. suis* outbreaks are mostly as a result of eating contaminated food and can be prevented by providing consumer education (Wongkamma *et al*., 2014).

Most people knew that they should not eat uncooked meat, but in this study more than 95% of infected cases claimed to still prefer to eat raw traditional dishes. A previous study on consumption behaviour for raw meat in the northern region concluded that changing people's behaviour was not possible since eating raw meat is a regional custom (Yothayai *et al*., 2006). Most of the people who eat raw meat claim do they like the delicious sweet flavour of raw meat. They also tend to eat raw meat with alcohol which might lower their inhibitions (Khadthasima *et al.*, 2007; Samerchea and Neungmek, 2008; Yothayai *et al*., 2006).

In the present study, some families misunderstood that only pork could cause foodborne disease and continued eating other kinds of raw meat. People in the outbreak area might avoid eating raw pork, but they simply changed to eat raw beef or raw fish instead which can cause cysticercosis and opisthorchis (Khamlar *et al.*, 2013). The Public health authorities have tried to implement risk communication materials such as posters and stickers in the villages, however, from our interviews most people were unware of these communication messages. Most people claimed to ignore this kind of communication materials since they cannot read well. Around 50% of the patients education in this survey was at primary level or lower, similar to the reported values from Thailand's national census. While the rate of literacy of the Thai population age over 15 years was 96.8% in 2013, the number of year in education for people in rural area was lower than for urban areas. People in rural areas aged 40 years and over were

had only 4-6 years of education and most did not complete primary school (National Statistic Office, 2014a).

A significant amount of the impact of *S. suis* infection was disability. Disability was reported in 33.36% of patients, of which 62.5% was hearing loss or deafness, 25% generalised weakness and 12.5% stiff, adhesive joints. Some patients with hearing loss or deafness claimed to be depressed due to difficulties in communicating, while in some cases the relatives said that the patients usually work on their orchards and rarely communicate with others. For the patients with generalised weakness, the patients and relatives said the patients could not undertake labour work. This had an impact on household income, since the patients had only primary education and could not find other work.

3.6 Summary

From 2002, all Thai citizen have been covered by health insurance guaranteeing them to access to health services. In general, there has been a significant increase in government health spending and a marked decline in out of pocket expenditure for patients. It is important that the budget evenly distributed and sufficient for each region.

The total *S. suis* DALYs amount to 129.81 or 7.41 per 100,000 population in Chiang Mai in 2013. The highest burden was found in males, at 3.5 times that for females. The age group 15-44 years, bore most of the burden (52.31%), followed by the 45-64 years age group (45.97%).

Most patients (72.72%) did not pay any for their treatment in hospitals and all treatment costs were covered by national health security scheme. The expenditure

falling to the national health security scheme was on average 37,955 baht (£759) per patients, ten times the per capita cost for the Universal Health Care Coverage of Thailand. This equates to an estimated 8 million baht (£160,000) for *S. suis* cases in Thailand per year.

Patients out of pocket costs were spent on transportation, and if appropriate any treatment costs at a private clinic and/or private hospital. Payment for transportation and for food for the relative(s) who took care of the patients while they were in hospitals made up most of the out of pocket costs at an average of 5,198 baht (£104) per patient.

S. suis also impacted on patients and their household. These impacts were mostly psychological, especially for patients with disabilities. Most patients with hearing loss or deafness were depressed due to difficulties in communication.

To decrease losses to the patients, their families and the health system incurred from *S. suis* infection, *S. suis* should be controlled in the food chain. Health education should be implemented to dissuade old and younger generations from eating uncooked animal products.

CHAPTER 4-Characterisation of swine *Streptococcus suis* in Chiang Mai, Thailand

4.1 Study Aims

The aim of this study was to characterise the pig production system in Chiang Mai province, estimating the prevalence of *S. suis* in backyard pigs and identifying the risk factors for *S. suis* infection.

4.2 Introduction

The Northern region of Thailand is endemic for *S. suis* infection in humans. Disease investigations confirmed that pigs were related to these outbreaks (Jaturpahu, 2009; Khadthasima *et al.*, 2007).

S. suis can often be present in a pig herd in the absence of any clinical signs of infection. There are several means of introducing *S. suis* into pig herds. The most common route of entry on to the farm is the introduction of apparently, healthy carrier pigs that are harboring *S. suis*. Within herds, the pathogen can be transmitted by droplets through the respiratory route. Stress factors, including high temperature, crowding, and poor management, may trigger the disease. Overcrowding and poor ventilation increase the chance of bacteria spreading.

S. suis can be either a primary or secondary pathogen. PRRS, *P. multocida*, *A. pleuroneumoniae*, *A. viridans* can increase its virulence (Pan *et al.*, 2016; Xu *et al.*, 2010; Gao *et al.*, 2016). Co-infected pigs exhibit more severe clinical symptoms and pathological changes, which leads to high mortality (Lin *et al.*, 2015).

Flies and rodents can also play a role in mechanical spread of the bacterium from improperly disposed infected carcasses (Neumann *et al*., 2009; Ramirez and Schwartz, 2009).

A few studies have investigated *S. suis* in pigs in Chiang Mai; Patungtod *et al* (2010) found the prevalence of *S. suis* in backyard pigs to be 10% (Padungtod *et al.*, 2010), while the prevalence of *S. suis* in healthy slaughtered pigs collected from slaughterhouses ranged from 8% to 18% (Lakkitjaroen *et al.*, 2009; Padungtod *et al.*, 2010). Eleven serotypes (2, 3, 4, 5, 7, 8, 9, 17, 21, 22 and 31) were isolated from the submaxillary glands of infected pigs, for sale at wet markets (Wongsawan *et al*., 2015). These studies mainly collected samples from pigs that were slaughtered and sold under the Animal Slaughtered Control and Meat Sale Act B.E. 2535 (1992).

The Animal Slaughtered Control and Meat Sale Act also permits home slaughter or slaughter at local slaughter sites under certain conditions, including, slaughter according to a religious ceremony and slaughter in remote areas as decreed by the provincial governor. Home slaughter and local slaughter sites are not overseen by veterinary inspection and backyard pigs slaughtered "at home" or at "local slaughter sites" present a risk for transmission of *S. suis* to humans.

In this chapter, the backyard pig holder system in was explored to understand backyard pig production in Chiang Mai. Backyard pigs were examined to determine the prevalence of *S. suis* and risk factors for infection.

4.2.1 Agriculture in Thailand

Thailand is predominantly an agricultural country. Agriculture in Thailand contributed 11.64% of GDP in 2014 (Anon, 2015a). Agricultural products are produced for domestic consumption and export. Exported agricultural products are a major source of income at almost 30% of total export value (Singhapreecha, 2014).

The Thai population was estimated at 65.125 million persons in 2014 (Anon, 2015b). Thirty-seven percent of the total population (24 million) are engaged in the agricultural sector (Agricultural census data report, 2013), accounting for 25.9% of households (5.9 million). There also has been an increase in the number (1.7%) and area (1.7%) of agricultural holdings over the during the past ten years.

There are two major agriculture activities: cultivation of crops and integrated crop-livestock farming. Of the total agricultural holdings, 96.4% are engaged in cultivation of crops, 76.5% in single activities and 19.9% with other agricultural activities such as livestock rearing and freshwater aquaculture. Of all of the holdings, 20.9% engaged in livestock production and 2.8% engaged only in livestock production. The major forms of livestock in Thailand are chicken, pigs and cattle (National Statistical Office, 2013).

4.2.2 Livestock in Thailand

Livestock production in Thailand has undergone significant changes over the last two decades in the pig, poultry and cattle sectors. Innovations such as new breeds, feed technology, housing, farm management and contractual arrangements have played important roles in Thai livestock development. Development of the livestock sector has also been driven by government regulations on farms and slaughterhouses, and through subsidies (Charoensook *et al.*, 2013). The pig and poultry industries are now Thailand's major industrial livestock sectors and the export value of livestock was over £2,000 million in 2015 (Anon, 2015c).

4.2.3 Pig production

The first pigs raised in Thailand by Chinese immigrants were local and native Chinese breeds including; Hainan, Kwai, Kradon or Rad, and Puang. Pigs were raised for consumption and as a second source of income (Youth Encyclopedia, 1994). The first imported exotic pig breed were black Essex pigs, imported from UK and recorded in the King Rama V era. These pigs were later raised in the Agricultural School in Nakorn Phatom in 1918. In 1939, Berkshire, Middle White and Tamworth pigs were imported from Australia. The Berkshire and Middle White pig breeds did not survive and were all dead by the end of the Second World War (Kamolnavin, n.d.). In 1948, the Department of Livestock Development Thailand imported, more Berkshire, Middle White and Tamworth pigs from Australia. Additional, Duroc Jersey, Berkshire and Hampshire breeds were imported from the United States in 1953, and Large White pigs were imported from UK in 1962 (Promthong, n.d.). All these pig breeds have been subsequently imported many times by both the Department of Livestock Development and the private sector. Most recently imported breeds are Large White, Landrace and Duroc.

Despite many attempts to improve pig stock in Thailand, intensive pig production is in its infancy, ongoing for only 30-40 years when the contract system was developed by feed mill companies, that provided piglets, animal feeds, drugs, veterinary services and farm management expertise to contracted producers (Charoensook *et al.*, 2013). From 1989, pig raising rapidly increased. In 2014, the pig population census indicated 9.5 million head (across all age groups) and annual production was 16.2 million head. There were 191,545 pig holdings, 95.01% of which

were small holdings (<50 pigs), and 0.13% pig farms with more than 5,000 pigs. The number of breeder pigs was approximately 1 million, 46% of which were owned by the two pig companies. Only 6.1% of the standing population are indigenous breeds (Anon, 2014b). Industrial pig farms raise commercial pig breeds, whereas backyard holdings raise either commercial breeds or indigenous breeds.

4.2.4 Veterinary services in Thailand

The Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives, is the national veterinary authority in Thailand. The DLD is responsible for: animal health; animal production; livestock extension; food safety of animal-derived products; veterinary public health; animal welfare; environmental impact of livestock farms and international animal health matters. This includes: prevention, control, and eradication of animal diseases; developing and increasing animal products in term of variety quantity, and quality to meet the national and international standards; encouraging scientific studies and research on animal production and health; and enforcing animal health laws and regulating livestock industries according to the laws (Department of livestock development, 2012).

Within DLD, there are officers from central, regional, provincial, and district levels, who provide services on animal-health and veterinary public health controls, and who undertake disease surveillance. Apart from DLD officers, sub-district livestock assistants and livestock volunteers also provide support relevant to basic animal health activities in cooperation with local administrative authorities at the sub-district and village level.

Thailand is geographically divided into nine livestock administrative regions (Figure 4-1), divided into 77 provincial livestock offices. Each provincial livestock office is sub-divided into 888 district livestock offices. Nine animal laboratories and the World Organisation for Animal Health (OIE) regional reference laboratory for FMD operate across these regions (Figure 4-2).

Chiang Mai province falls within livestock administrative region 5, which is subdivided into 25 sub-district livestock offices. Chiang Mai has the sixth largest pig population in Thailand of 335,716 head in 2011. Chiang Mai also has the largest number of holdings at 16,256 (8.49%) (DLD, 2011). The number of holdings with less than 50 pigs was 16,000 and only 256 holdings held over 50 pigs (DLD, 2011).

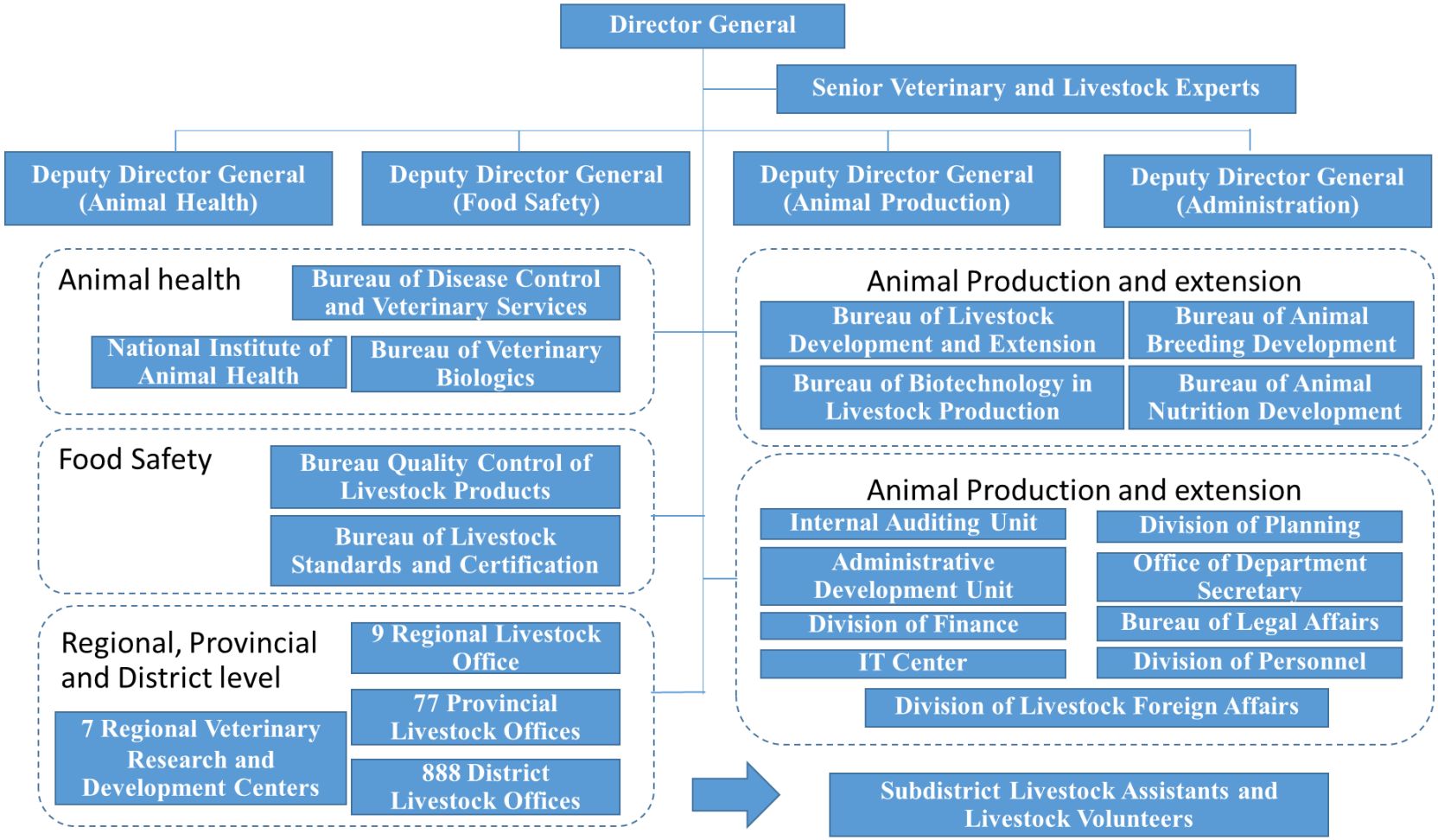


Figure 4-1 Structure of Thailand's Department of Livestock Development (DLD); Source: www.dld.go.th

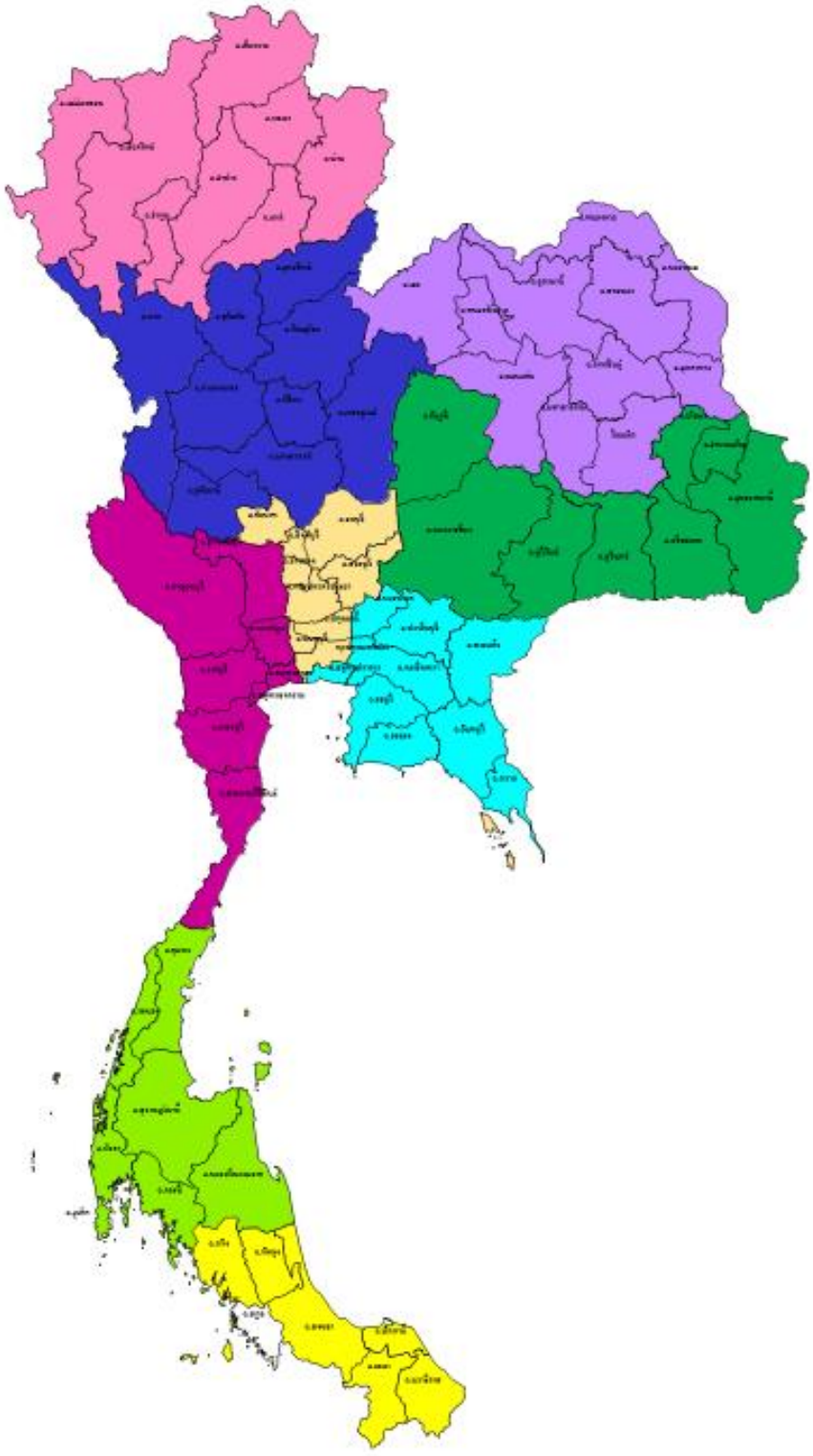


Figure 4-2 Map of Thailand divided into nine administrative livestock regions

4.3 Methodology

4.3.1 Study method

A cross-sectional study was undertaken in backyard pigs in selected villages in Chiang Mai. Backyard pigs were defined as pigs in holdings of 30 pigs or less. Villages were the primary sampling units and selection of pigs was done randomly. The samples were collected between November 2013 and June 2014.

4.3.2 Sampling plan and sample size determination

A two-stage cluster sampling methodology was used. Two-stage cluster sampling is used when it is impossible to create a complete list of all herds. Thus, it is likely to be a list of places where animals are known to be kept (villages) from which a set number of villages are randomly selected. A simple case of two-stage sampling is obtained by selecting clusters in the first stage and then selecting a sample of elements from every sampled cluster. In this study, in the first stage, villages were selected using cluster sampling method by C-survey version 2.0 (Farid and Frerichs, 2007). In the second stage, backyard pigs were selected using simple random sampling from each sampled cluster.

Since the manpower and budget were limited, C-Survey version 2.0 (Farid and Frerichs, 2007) was used to test an acceptable sample size. To test the proposed sample size for cluster sampling, the expected prevalence and design effects need to be known. Actual values for these were not known and were assumed as follows; based on 13% average prevalence of *S. suis* survey in Thailand obtained between 2009-2011 (Padungtod *et al*., 2010b; Lakkitjaroen *et al.,* 2009; Lakkitjaroen *et al*., 2011), and an expected prevalence of 15% was used as it gives a larger sample size. In addition, there

was no previous information about the design effect for *S. suis*, so an estimate of "medium" i.e. equal to 4.0 was used. The design effect is a measure of how much greater the variance is of a cluster survey than a similar-sized group where data is collected as a simple random sample.

Article	Prevalence (%)	Number of sample
Lakkitjaroen (2009) in Thai	18.06	72
Padungtod (2010)	9	212
Lakkitjaroen (2011)	18.1	72
Weight Average	12.67	

In the first stage of sampling, C-survey version 2.0 was used to select the villages (primary sampling unit) that make up each cluster. A list of villages and number of pigs in each village was obtained from Chiang Mai provincial livestock office, and a total of 25 villages were sampled at the first stage with probability proportionate size (PPS). Based on these assumptions, an expected prevalence was to be estimated with a desired absolute precision of ±8% with an attribute that has an estimated 95% confidence limit of 7.4% to 22.6%. Hence, the proposed of 25 clusters and 15 pigs per cluster was acceptable for the survey. In the next stage, 15 pigs were randomly selected from each sampled cluster. For the survey of *S. suis* in backyard pigs, all pigs aged ≥3 weeks and over in villages were the sampling units. The total pig sample in this study was 375.

4.3.3 Data and Sample collection

4.3.3.1 Tonsil swab

Tonsilar swabs were taken for each pig. To collect the samples, pigs under 15 kg were restrained by holding the pig's head by the ears or restraining it by a noose

then opening the mouth using a gag. Pigs over 15 kg were restrained using a snare. The mouth was opened by placing a bar of the gag above the tongue. The tonsil is observed as a soft palette at the rear of the throat. It can be distinguished from the salivary glands by a dimpled appearance and rough surface. The surface of the tonsil was scratched with a cotton swab several times to exude a mucosal excretion from the crypts. The cotton swab was place in Stuart transport medium and kept at 4ºC until processed.

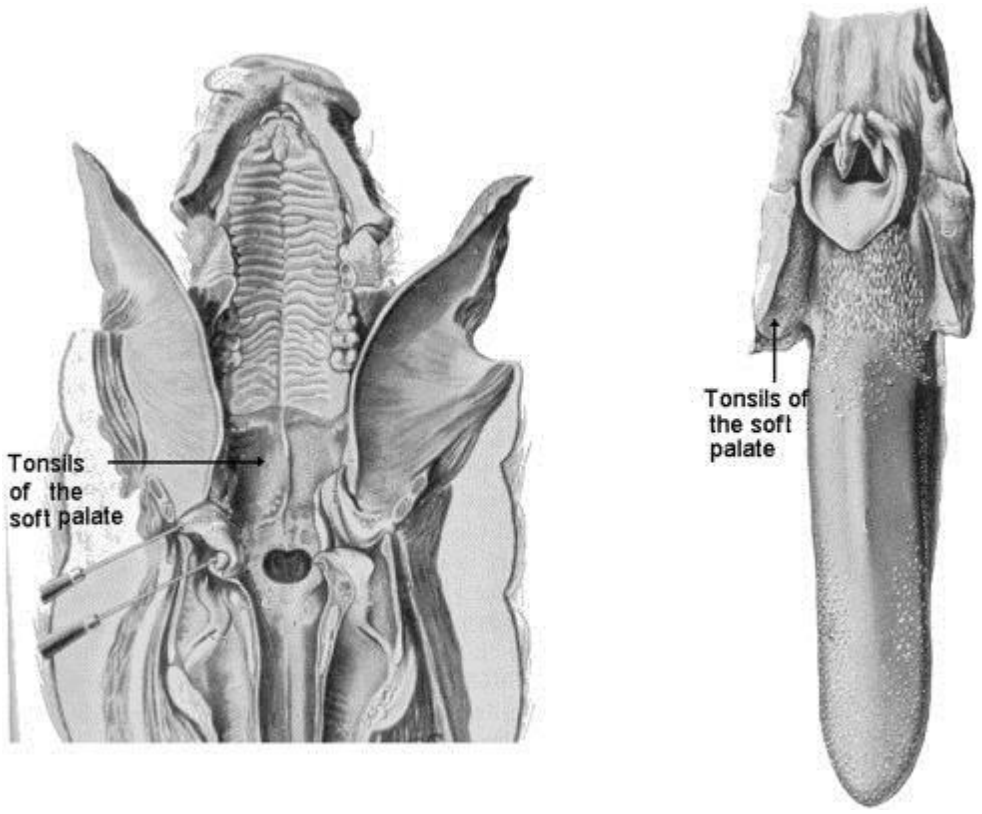


Figure 4-3 Oral cavity and tongue of a pig presenting the tonsils of the soft palate (Nickel et al., 1979 as adapted by Vieira-Pinto et al., 2012)

4.3.3.2 Data collection

Face-to-face questionnaire surveys were performed to collect data on pig production characteristics and management. Aspects covered included: farm size, farm management practices, housing system, and diseases and control practice.

4.3.3.3 Laboratory tests

Bacterial isolation was performed at the Veterinary Research and Development Center (Northern region), Lampang, Thailand following standard protocol, as follows.

Bacterial culture

S. suis was isolated from palatine tonsil swabs. The cultures were stabbed and streaked onto a blood agar plate incubated at 37^{o}C and 5% CO_2 for 24 hours. The stab region (Figure 4-4) was used to differentiate *S. suis* from O_2-labile hemolytic *Streptococcus,* which grows better in anaerobic conditions. If the stab region shows β-hemolysis, the bacterium is **not** *S. suis*. Suspected *S. suis* colonies (Figure 4-5), which were small round, 0.5-1.0 mm, mucoid, transparent and exhibited α-hemolysis, were selected for further confirmation.

Gram stain

In this study, the presumptive isolates were stained by Gram's stain method and observed under light microscope. Streptococci are gram-positive cocci, occurring in pairs or chains.

Biochemical confirmation

S. suis was identified as being negative to a catalase and Voges-Proskauer (VP) test, and positive to Esculin hydrolysis. The tests on sugar consumption give positive to trehalose and insulin but negative to sorbital and mannitol.

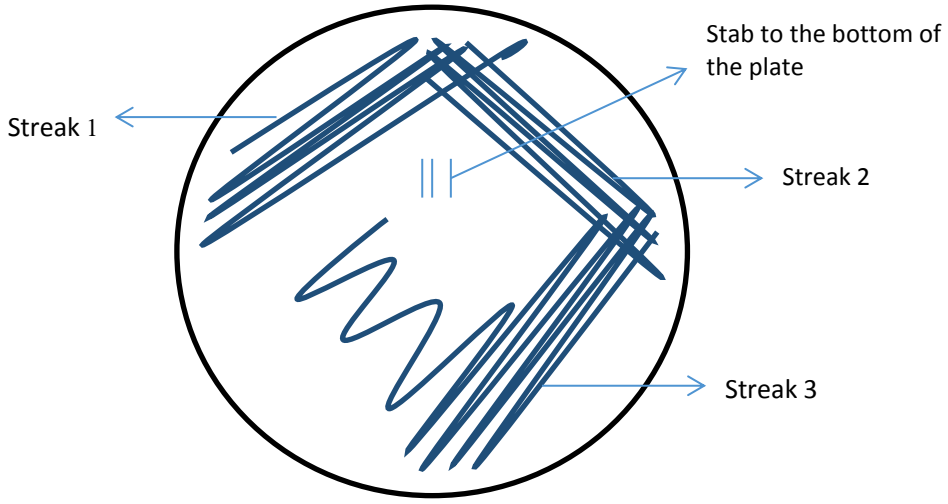


Figure 4-4 The streak series to isolate *Streptococci* spp. on blood

Figure 4-5 Gray-whitish, α-hemolytic colonies of Streptococcus suis on sheep blood agar plate at 35^{o}C in the presence of 5% CO_2 after 24 hr incubation

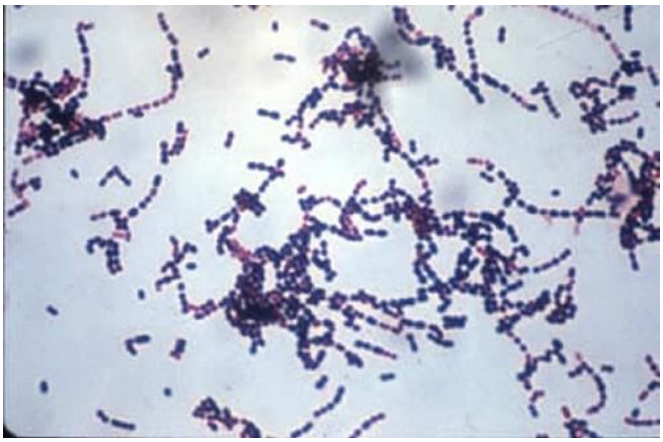


Figure 4-6 *Streptococcus suis,* broth culture, Gram's stain. (Merckmanuals.com in courtesy of Dr. John Prescott)

4.3.4 Statistical analysis

All data were entered into Microsoft Excel. Analyses were performed using WinPepi (Abramson, J.H., 2011). Percentages and 95% confidence intervals were used to describe the characteristics of backyard pig production.

The prevalence (*P*) of *S. suis* in pigs was estimated according to Cochran's procedure using equal-sized clusters (Abramson, 2011). The 95% confidence interval and design effect were reported. The point estimate of the prevalence is the ratio of the total number with the attribute under study to the combined number in the clusters.

The formula for calculating prevalence is;

$$P = \frac{\textit{number of individual having a disease at a point in time}}{\textit{number of individual in the population at risk at the point of time}}$$

The 95% confidence limits are computed as

$$P \pm t(\mathrm{SE})$$

where P = prevalence

t = the two-tailed critical value of Student's *t* at alpha = 0.5, with (C-1) degree of freedom

C = number of clusters

SE = standard error

Cross sectional analysis was performed to find the association of epidemiological characteristics of *S. suis* infection. To identify a confounding factor, the Mantel-Haenszel test of stratifying variables generates an estimate of an association controls for confounding effects presented as adjusted odd ratio. Heterogeneity was accessed using Higgin's & Thomsom's H. The summary results were presented as odd ratio and 95% confidence intervals.

4.4 Results

The survey was carried out in 25 villages within 15 districts of Chiang Mai province. The number of sampled holdings in each village is shown in Table 4-1 and Figure 4-7 Map of Sampled villages in Chiang Mai province. A total of 114 holdings were explored and interviewed.

Table 4-1 Name of the sampled villages and number of sampled holdings

Village	Sub-district	District	No. of sampled holdings
Wang Tarn	Luang Nua	Doi Saket	4
Papai	Mae Pang	Doi Saket	3
Huay Jo	Sop Tia	Chom Thong	5
Mae Ja	Thung Khao Phuang	Chiang Dao	6
Tha Lo	Doi Lo	Doi Lo	3
Rai Pattana	Doi Lo	Doi Lo	3
San Kamok	Pa Tum	Praw	4
Pa Nai	Pa Nai	Phrao	6
Mae Pang	Mae Pang	Phrao	2

Huay Khao Lip	Mae Win	Mae Wang	13
Nong Tao	Mae Win	Mae Wang	10
Mae Sapok	Mae Win	Mae Wang	9
Pa Ngew	Ta Nua	Mae On	11
Nam Cham	Rong Wua Dang	San Kamphaeng	3
Mae Sa Noi	Pong Yang	Mae Rim	7
Nong Hoi kaw	Mae Ram	Mae Rim	1
San Pranet	San Pranet	San Sai	3
Dong Pa Sang	Makun Wan	San Pat Tong	3
San Hao	Ban Glang	San Pat Tong	3
Dong Pa Nhiew	Makhun Wan	San Pat Tong	2
Kong King	Nong Kwai	Hang Dong	2
Pa Hang	Sop Poeng	Mae Taeng	3
Pang Muang	Sop Poeng	Mae Taeng	3
Sop Lerm	Ban Pao	Mae Taeng	3
Nong Baen	San Sai	Saraphi	2

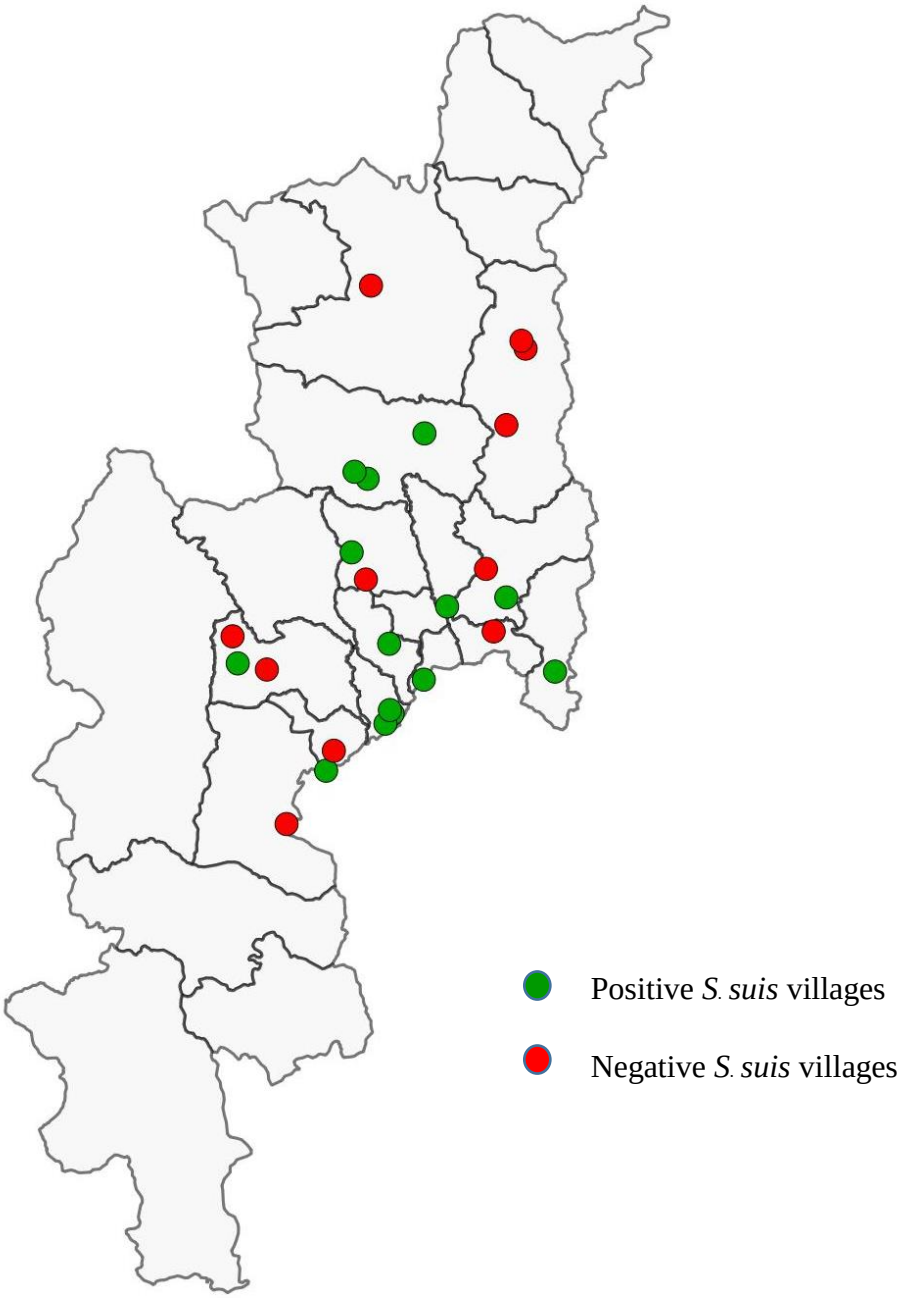


Figure 4-7 Map of Sampled villages in Chiang Mai province

4.4.1 Characteristics of backyard pig holdings in Chiang Mai

4.4.1.1 Characteristics of pig holding owners

Almost all pig holding owners were agriculturists. Most had graduated at primary level (50%), followed by lower than primary level (36%), secondary level (10.5%), and diploma level (3.5%). Twenty-one per cent of the pig holdings in this study raised pigs as a major source of income, whereas the other 79% reared pigs as a secondary income source, along with other main agricultural products. The holdings that reared pigs for secondary income were mostly managed by housewives.

From the interviews, backyard pigs raised as a secondary income source were generally not raised continuously. Pigs were reared when the market price increased, and pig keeping ceased when the price dropped. Of the holdings in this present study, 62.3% had raised pigs between 1 to 10 years, 35.9% for more than 10 years, and the other 1.8% just raised pigs for 2-3 months.

4.4.1.2 Holding characteristics

Holding size

Backyard farmers mostly aimed to raise pigs for meat or to provide an additional source of income. Holdings with sows and fatteners had the highest share (39.5%), followed by only fattening holdings (30.7%), and holdings with boars, sows and fatteners (11.4%).

The median number of pigs per holding was 7.5 head, ranging from 1 to 30 head, (30 head is the highest number for backyard holdings according to the Department of Livestock Development, Thailand). Most backyard pigs were reared as secondary income sources, of which 52.6% had one to five pigs per holding.

Table 4-2 Number of holdings by farm categories and size

Holding category and size	No. of holdings	%	95% CI
Category			
Boar, sow and fattening	13	11.4	6.8-18.5
Boar and fattening	7	6.1	3.0-12.1
Sow and fattening	45	39.5	31.0-48.7
Only boar	6	5.3	2.4-11.0
Only sow	8	7.0	3.6-13.2
Only fattening	35	30.7	23.0-39.7
Pigs per holding			
1-5	60	52.6	43.5-60.2
6-10	20	17.5	11.7-25.6
11-15	7	6.1	3.0-12.1
16-20	4	3.5	1.4-8.7
21-25	6	5.3	2.4-11.0
26-30	17	14.9	9.5-25.4

Pig breed

The backyard holdings reared either native-breed or commercial-breed pigs. Around half of the backyard holdings in Chiang Mai raised native-breed, exotic-breed, and mixed-native or exotic with commercial breed pigs. The exotic pigs, for example Meishan and Hainan, are native to southern China. Mixed breeds, between exotic and commercial pigs have been promoted to backyard pig holders by the Department of Livestock Development. The remaining backyard holdings raised commercial breed pigs, such as Duroc, Landrace, and Large Whites. Many raised both native or exotic breeds together with a commercial breed.

Housing

The size, shape and material of pig pens varied in design. Most of the pens in this study had a concrete floor (81.6%). Dirt-floor, traditional pit and high-floor wood

houses accounted for 15.8% of constructions. All pig houses were open for natural ventilation, with curtained sidewalls to prevent sunlight and rain. The number of holdings according to each housing type is given in Table 4-3. Several holdings (2.6%) did not have pig housing; instead, pigs were tethered in the open space beneath their houses. The median space for one pig was 1.2 square meter (minimum=0.25, Q1=0.75, median=1.20, Q3=2.00, maximum=4.50).

Table 4-3 Number of holdings by housing types

Housing type	No. of holdings	%	95%CI
Concrete floor	93	81.6	73.5-87.6
Dirt floor	12	10.5	6.1-17.5
Pit house	5	4.4	1.9-9.9
High floor wood house	1	0.9	0.2-4.8
Tying under house	3	2.6	0.7-7.5

4.4.1.3 Holding management characteristics

Source of pig

The sources of pigs in backyard holdings included: pigs bred in the holding; pigs purchased from another small holding within the village and pigs from commercial farms in the area, governmental farms, or university farms (Table 4-5). The Governmental farms include the Livestock Research and Breeding Center, the Royal Project, and the Royal Development and Study Center. This study found that most pigs were bred on the holdings (46.49%) or were obtained from other holdings within the village (31.72%).

Table 4-4 Types of backyard pig house (clockwise from top left: concrete floor, dirt floor, pit house, high-wood floor house)

Table 4-5 Number of holdings by source of pig

Source of pig	No. of holding	%	95% CI
Bred on holding	53	46.49	37.6-55.6
Bred within village	43	37.72	29.4-46.9
Nearby villages	2	1.75	0.5-6.2
Government organizations	16	14.04	8.8-21.6

Pig feed and water use

Both self-mixed and commercial feed were used to feed pigs in the study holdings (Table 4-6). About 62% of holdings used self-mixed feed from agricultural products, nearly twice as many holdings than those using commercial feed only. Agricultural products fed to pigs included: rice bran, broken rice, soya bean, maize, banana stalk and bud, and leftover food. The holdings that used only commercial feed comprised 36.8%, whereas 0.9% used both self-mixed and commercial feed.

Most of the water used in backyard holdings (70.2%) was obtained from the public water supply. In remote areas where there was no public supply, natural underground (21.9%) and over ground natural water sources, such as rivers or canals, (2.6%) were used and 5.3% collected and used rainwater.

Table 4-6 Number of holdings by feed and water used

Source	No. of holdings	%	95% CI
Feed			
Self-mixed feed	71	62.28	53.1-70.6
Commercial feed	42	36.84	28.6-46.0
Both self-mix and commercial	1	0.88	0.2-4.8
Water			
Water supply	80	70.18	61.2-77.8
Underground water	25	21.93	15.3-30.4
Overground water	3	2.63	0.9-7.5
Rainwater	6	5.26	2.4-11.0

Cleaning and disinfection

The cleaning frequency was shown in Table 4-7. Most of the pig houses with concrete floors were cleaned one to three times daily; some were cleaned 1-3 times a week, or once per month. However, around 44% had never cleaned the pig pens. None of the holdings used disinfectants to clean the pens.

Table 4-7 Number of holdings by cleaning frequency

Cleaning frequency	No. of holdings	%	95%CI
At least once per day	43	46.24	36.5-56.3
At least once a week	9	9.68	5.2-17.4
Never cleaned	41	44.09	34.4-54.2

Deworming and Vaccination

All backyard holdings in Chiang Mai province had similar management practices in terms of deworming and vaccination. Deworming and vaccination were not commonly used in the backyard holdings. Pigs were dewormed when drugs were provided by the livestock officers (not often) and some households never dewormed their pigs. Vaccination was implemented only if it was provided by livestock officers. Most backyard pigs had never been vaccinated. The owners did not know what vaccines the pigs were given. The livestock officers normally provided classical swine fever vaccine.

Using of personal protective equipment

Most holdings did not use personal protective clothing and equipment to prevent disease infection. Some farmers sometimes used boots. None were aware that *S. suis* could be transmitted by close contact to pigs or pig carcasses.

4.4.2 Prevalence of *S. suis* in backyard pigs in Chiang Mai

In total, 114 holdings in 25 villages participated in this survey. A total of 375 tonsil swab samples were collected in the sampled villages. *S. suis* was isolated from 18 out of 375 samples by bacterial isolation and PCR. The overall *S. suis* prevalence from tonsil swab samples was 4.8% (95% CI: 2.2-7.4) (Table 4-8). Positive samples came from 11 out of 25 villages. *S. suis* positive pigs were identified on 15 holdings.

Table 4-8 Prevalence of *S. suis* in backyard pigs in Chiang Mai

Level	Number examined	Number positive	Prevalence (%)	95% confidence interval
Chiang Mai	375	18	4.8	2.2-7.4
Village	25	11		

4.4.3 Risk factors of *S. suis* in backyard pigs from the case-control analysis

Several factors that can impact on *S. suis* infection in pigs including: overcrowding, ventilation and stress from improper management. The backyard holdings in this study all exhibited similar management practices and all had open ventilation. The only factor that may be associated with *S. suis* infection was space given to the pigs. The median space given per pig in *S. Suis* positive holdings was 0.79 square meter per head, lower than the median of 1.38 square meter per head in *S. suis* negative group (**Error! Reference source not found.**). However, the medians of positive and negative groups cannot be compared as the data distributions were dissimilar (Figure 4-8)**Error! Reference source not found.**.

Table 4-9 Factors associated with *S. suis* in backyard pigs

Parameter	Positive Median (95%CI)	Negative Median (95%CI)
Pen space per pig	0.79	1.38

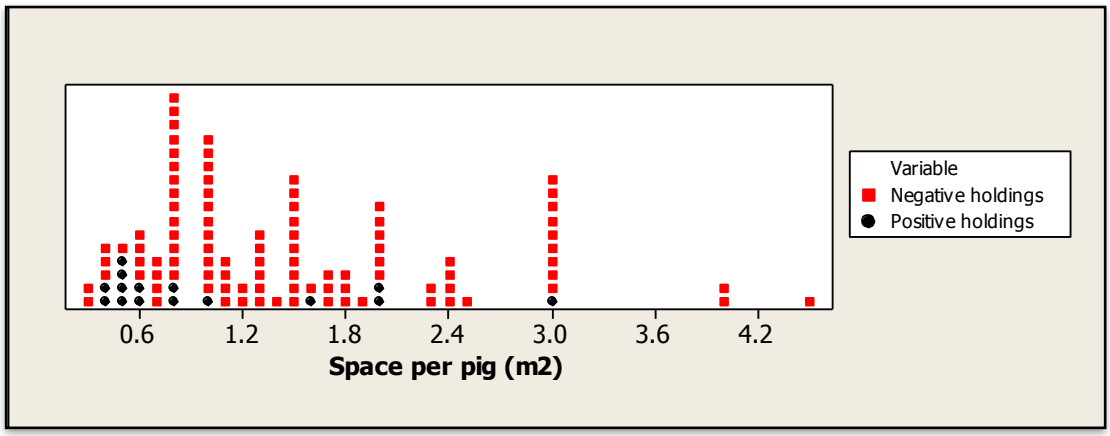


Figure 4-8 Dot plot of holding by pen space (m^2) per pig

Case-control analysis, shows a significant positive association of space given per pig of less than or equal to 1.2 m^2 and *S. suis* infection. The odds ratio suggested that pigs living in spaces lower than 1.2 m^2 had a higher risk of *S. suis* infection (4.35 times than those living in larger space), with the Fisher's 95% confidence interval was between 1.07 and 25.21.

To examine if a concrete floor could be confounding the association of *S. suis* infection, a stratified analysis was performed. The odds ratio of the pen space per pig, adjusted by presence and absence of concrete floor was 0.46, with the Fisher's 95% confidence interval was between 0.15 and 1.42 (Table 4-10). The results suggest that no confounding was identified. Moreover, Higgin's &Thompson's heterogeneity had a value of 1.2, therefore, there was no evidence of interaction between pen space per pig and concrete floor type of housing.

Table 4-10 Association of epidemiological characteristics between *S. suis* infection by case-control analysis.

Parameter	Odds ratio	95%CI
Pen space per pig (≥1.2 m2, <1.2 m2)	**4.35**	**1.07-25.21**
Pen space per pig, adjusted by concrete floor (yes, no)	0.46	0.15-1.42

4.5 Discussion

Chiang Mai is endemic for *S. suis*. The common route of infection is from human consumption of raw or under-cooked pork or pig-blood. Traditional dishes in the northern provinces of Thailand, including Chiang Mai, include raw or under-cooked meat, hence the high *S. suis* incidence reported in this region (Bureau of Epidemiology, 2013). Chiang Mai also has the highest pig density in the northern part of Thailand (DLD, 2011). Few studies have examined *S. suis* studies in pigs in this region and most have focused on commercially slaughtered pigs (Chanto, 2013; Lakkitjaroen *et al.*, 2009). Only one study examined *S. suis* in backyard slaughtered pigs (Padungtod *et al.*, 2010). The current study aimed to investigate *S. suis* in live backyard pigs, exploring the production and management system and risk factors for infection.

4.5.1 Backyard pig production system in Chiang Mai

In Thailand, a pig farm refers to holdings that have more than 30 pigs (Bureau of Livestock Standards and Certification, 1999). Backyard holdings have less than 30 pigs. From the pig census in 2013 (unpublished data), Chiang Mai had 16,119 pig holdings with a total of 202,841 head, of these, 389 were backyard holdings of 76,327 head. Chiang Mai is the province with the highest numbers of backyard pigs in

Thailand. Backyard holdings generally raise pigs for consumption and to supplement income. Backyard pigs were not raised on a continuous basis, but are raised according to the price of pork in the market. The numbers of pigs kept are constantly changing. Most backyard pigs were reared for a supplementary source of income. The median holding size in this study was 7.5 pigs per holding (all ages of pig). Most of the holdings reared sows and fattening pigs or only fattening pigs. Over half (52.6%) of the holdings had 1-5 pigs. Pig breeds were a mixture of native and exotic breeds. Most holdings used agricultural products and household leftovers to feed the pigs. Pigs were not commonly dewormed or vaccinated. This was in contrast to a study in Nan, a small province in northern Thailand where Kongkaew (2011) observed 60% and 27% of pig holdings in Nan using dewormers and vaccines, respectively.

Around 80% of pig-housings had a concrete floor, the preferred type of housing since it is easy to build and clean (Division of Livestock Extension and Development, n.d.). In this study, there were no free-range pig holdings. By contrast, studies in the 1970s and earlier showed that most pigs raised in the highland area in Thailand were free-range scavengers (Falvey and Visitpanich, 1980). Free-range scavenging was also observed in 2010 in the highland area (Kongkaew, 2011). Free-range scavenging is rarely seen nowadays since the Department of Livestock Development have been promoting and helping to build appropriate pig housing. Around 40% of the concrete floor pig housings were never cleaned. These holdings used rice-husks to absorb pig manure and urine, and the bedding generated a manure-litter mixture. Once the pigs were sold, the manure-litter mixture was sold as an organic fertilizer.

4.5.2 *Streptococcus suis* prevalence

This study found 18 *S. suis* positive pigs from 375 sampled pigs in Chiang Mai, giving an estimated prevalence of *S. suis* in backyard pigs 4.8%, with 95% confidence intervals between 2.2% and 7.4%. In previous studies, the prevalence of *S. suis* in slaughtered pigs in Chiang Mai was reported between 9-38% (Chanto, 2013; Lakkitjaroen *et al.*, 2009; Padungtod *et al.*, 2010). In other northern provinces, the prevalence of *S. suis* was reported as 4-73% from slaughtered pigs (Lakkitjaroen *et al.*, 2009; Neungmek and Pathanasophon, 2011). The high prevalence (73%) was reported from Phayao province, which had large *S. suis* outbreak in 2007.

There are three reasons that might explain why pigs in previous studies have higher prevalence than the current study. Firstly, the majority of the pigs in previous studies were raised in commercial farms. In intensive farming, pigs have a higher chance of bacterial transmission due to poor ventilation in housing, especially in summer and rainy season, improper management of weaning pigs, and other operations that can cause stress to pigs such as tail docking, teeth clipping, earmarking and tattooing and intensive vaccination program. Secondly, pigs may be stressed during transport to the slaughterhouse and improper lairage period management may be a factor influencing bacterial transmission and growth. Neungmek and Pathanasophon (2011) reported that pigs kept in crowded cages, had 1.3 times more *S. suis* than pigs in proper lairage pens. Lastly, in previous studies, *S. suis* was directly isolated from the tonsils. Isolation of *S. suis* directly from the tonsils offers the best higher opportunity to find *S. suis* (Clifton-Hadley *et al.*, 1984). Nevertheless, the tonsil

swab, the technique used in this study, is the most appropriate method for sample collection from a live animal (Lakkitjaroen *et al.*, 2009).

4.5.3 Risk factors

Since most of the backyard pig holdings had similar characteristics and practices, there were only few management factors that could be associated with *S. suis* infection. The most important management risk factor was overcrowding; *S. suis* circulate in the air and if there was poor ventilation in the housing, there was a higher chance of transmission of *S. suis* to other pigs in the same pen. This study found that pigs living in larger spaces (≥ 1.2 m^2) had lower chance 4.35 times of *S. suis* infection.

Most holdings in this study had a concrete floor. *S. suis* have been shown to survive for less than four hours on concrete surface (Dee and Corey, 1993). Concrete floors lower the risk of *S. suis* transmission to other pigs, especially if regularly cleaned and disinfected. A concrete floor was not a confounding factor in this study.

4.6 Summary

Chiang Mai province is endemic for *S. suis* and contaminated pork is a major source of human infection. The local practice of eating raw traditional raw pork dishes in this area is a risk factor for infection. Backyard pig production in Chiang Mai province was mainly to provide a secondary income. Most holdings raised sows and fattening pigs or fattening pigs alone. Pigs were sourced mainly from other holdings within the village. Most pigs were kept in pens and free roaming pigs were rarely found. The overall prevalence of *S. suis* in backyard pigs in this study was 4.8%, substantially lower than the prevalence reported in commercial pigs from previous studies. Overcrowding was identified as a risk factor for *S. suis* spread.

CHAPTER 5-Molecular epidemiology of swine *Streptococcus suis* in Chiang Mai, Thailand

5.1 Study aims

This study aimed to investigate the population biology of *S. suis* in Chiang Mai Province by genotyping isolates to reveal the molecular epidemiology of *S. suis* using Multilocus Sequence Typing (MLST).

5.2 Introduction

Genetic diversity studies have become popular in the field of molecular epidemiology of bacteria. Microbial typing analyses the relationships between isolates to determine the source and routes of infections, confirm or rule out outbreaks, trace transmission of healthcare-associated pathogens, recognize virulent strains and evaluate the effectiveness of control measures (Ranjbar *et al.*, 2014). Several epidemiological studies of serotyping and genotyping of *S. suis* have been undertaken revealed the diversity of *S. suis*. Most studies of *S. suis* diversity in Thailand have involved serotyping; only a few studies have involving genotyping of *S. suis* isolates from humans, slaughtered pigs, and diseased pigs. Data regarding *S. suis* isolates serotyping and genotyping from backyard pigs in Thailand are not available. In this study molecular identification of the capsule polysaccharide genes and the genotyping assessment of *S. suis* in backyard pigs was undertaken to provide insights into the epidemiology of this *S. suis* in Thailand.

5.2.1 Molecular epidemiology

Molecular epidemiology is recognized as a multi-disciplinary aspect of epidemiology and medical science that incorporates the use of molecular and other biologic measurements in epidemiology (Foxman and Riley, 2001). It provides the understanding on the distribution of disease in populations and identifies the

etiological determinants of these relationships (Snow, 2011), making available information that can be used to implement prevention and control measures to improve the health of populations (Slattery, 2002). Although the most published studies have focused on cancer, other pathogenic agents have been widely studied (Slattery, 2002) including; tuberculosis, Malaria, *Neisseria spp*., *Cryptosporidium spp*. and *Staphylococcus* aureus (Asma *et al.*, 2015; Doudoulakakis *et al.*, 2016; Hoza *et al.*, 2016; Kateera *et al.*, 2016).

The study of genetic diversity of bacterial populations defines diversity in terms of mutation, genetic drift and selection of species, enabling the relatedness between isolates to be established. Information on the population structure and the epidemiology of different bacteria groups can support epidemiological investigations (Carricoteam, Sabat and Romirez, 2013).

5.2.1.1 Serotyping

Serotyping identifies *S. suis* strains based on their capsular polysaccharide. However, there are *S. suis* isolates that do not agglutinate with any of the 35 typing antisera available which are classified as a non-typable. Recently, protocols for serotyping using PCR to amplify serotype specific *cps* genes have been developed, that cover all *S. suis* serotypes, and have been widely employed by many laboratories (Kerdsin *et al.*, 2014; Liu *et al.*, 2013; Okura *et al.*, 2013). Some isolates considered non-typable isolates by agglutination techniques can be identified by multiplex PCR across the 35 serotypes (Okura *et al.*, 2014). Commercial kits are available for identification of serotype 2 and can be used for both human and pig suspected cases.

Nine serotypes (2, 4, 5, 9, 14, 16, 21, 24, 31) of *S. suis* have been identified from humans (Goyette-Desjardins *et al*., 2014; Haleis *et al*., 2009; Hatrongjit *et al*., 2015; Kerdsin *et al*., 2009, 2015; Nghia *et al*., 2008; Zhu *et al*., 2013). Serotype 2 is the most dominant, followed by serotype 14 (Goyette-Desjardins *et al.*, 2014).

In diseased pigs, globally the predominant *S. suis* serotypes are serotype 2, 9, 3, ½ and 7, in decreasing order (Goyette-Desjardins *et al.*, 2014). In North America, serotype 2, ½ and 3 predominante in descending order (Messier, Lacouture and Gottschalk, 2008). Serotype 2 accounts for more than 40% of samples from diseased pigs in China (Wei *et al.*, 2009). In Europe, although serotype 2 is the most prevalent in France, Italy and Spain, serotype 9 is the most common serotype in Belgium, the Netherlands and Germany (Wisselink *et al.*, 2000). In the United Kingdom serotype 1 and 14 play important roles (Goyette-Desjardins *et al.*, 2014).

In Thailand, the dominant serotype in both humans and pigs is serotype 2, but serotype 5, 14 and 24 have been reported in *S. suis* from humans, and serotype 1, 2, ½, 3, 4, 5, 7, 9, 14, 16 and 19 have been identified from pigs (Chanto, 2013; Kerdsin *et al.*, 2011, 2012; Maneerat *et al.*, 2013; Takamatsu *et al.*, 2008).

5.2.1.2 Multilocus sequence typing (MLST)

Many non-molecular and molecular tools have been developed for examining allelic variation for population genetic analysis. Non-molecular technique including antibiogram, biotyping, serotyping, bacteriophage typing and bacteriocin typing are labour intensive and time-consuming to be practical for epidemiological investigations (Ranjbar *et al.*, 2014). Nucleic acid based methods include plasmid DNA restriction analysis, hybridization techniques, chromosomal DNA restriction analysis, pulsed

field gel electrophoresis (PFGE), PCR-based subtyping methods, and multilocus sequence typing (Quinn *et al.*, 2015).

Among the molecular typing methods, MLST, is the most widely used technique for indexing allelic variation for population genetic studies. MLST data have been employed in both epidemiological surveillance at various scales and in a fundamental studies of pathogen biology, such as the population biology, and evolution of bacteria (Urwin and Maiden, 2003).

MLST was first developed for *Neisseria meningitidis* in 1998 (Maiden *et al.*, 1998). MLST is a DNA sequence based technique for typing of multiple loci, characterising isolates of bacterial species using the sequences of internal fragments of multiple house-keeping genes (Maiden, 2006). MLST can detect more variation, resulting in more alleles per locus than previous techniques, and the resulting sequence data can be compared readily (Maiden *et al.*, 1998). MLST is also considered the gold standard of typing for many bacterial species, for example *Neisseria spp*., *Streptococcus pneumoniae, Helicobacter pylori* (Larsen *et al.*, 2012).

MLST analyses internal nucleotide sequences of approximately 450 to 500 bp of multiple housekeeping genes (usually seven). For each gene, the different sequences present within isolates are assigned as a distinct allele. The alleles for each of seven loci define the allelic profile or sequence type (ST) for each isolate (Fratamico, Bhunia, and Smith, 2005). MLST sequence data are unambiguous and the allelic profiles of isolates can easily be compared (Maiden *et al.*, 1998). MLST involves PCR amplification using specific primers for the target locus followed by DNA sequencing.

Allele number and sequence type are assigned to the allelic profile generated and these are compared to find the relatedness of isolates.

An MLST protocol was developed for *S. suis* in 2002 (King *et al*. 2002) using seven house-keeping genes; *aroA, cpn60, dpr, gki, recA, thrA and mutS.* This method is used to determine the sequence type (ST) of *S. suis* strains. At least 94 MLST databases, allele sequences and ST profiles, are available in on line database of which 84 are for bacteria, http://pubmed.org (Larsen *et al.*, 2012) to ensure a uniform nomenclature applied across studies.

5.2.2 *S. suis* sequence type

S. suis ST from pigs and humans obtained between 2002 to 2013 have been reviewed (Goyette-Desjardins *et al.*, 2014). ST1 is responsible for a high proportion of *S. suis* disease in humans worldwide and these ST have been mostly recovered from *S. suis* serotype 2 isolates. ST1 is the most predominant ST in Europe, Asia and South America, associated with *S. suis* disease in both pigs and humans. In South America ST1, predominates with reports from human cases in Argentina and French Guiana (Goyette-Desjardins *et al.*, 2014).

In Europe, the most STs determined from clinical isolates belongs to the ST1 complex (Blume *et al.*, 2009; King *et al.*, 2002). In addition, the ST20 complex is also high among the strains isolated from humans in the Netherlands (Schultsz *et al.*, 2012).

In North America ST28 and ST25 were equally the most dominant ST in isolates from diseased pig. ST28 is most frequesnt strains from in Canada (75%). ST25 has also been identified from diseased humans in North America (Fittipaldi *et al.*, 2011). ST1 which has higher virulence than ST25 and ST28 is found in North America,

but at a low prevalence (Fittipaldi *et al.*, 2011). The majority of the isolates from humans with endocarditis in Japan were assigned to ST1 and ST28 complexes, while the isolates from pigs with endocarditis were classified into ST1, ST27 and ST28 complexes (Onishi *et al.*, 2012).

In Asia, not many STs have been reported from diseased pigs. In China ST7 is the most predominant in diseased pigs followed by ST1 of 77% and 22% respectively, and most of strains are assigned to CC1 (Chen *et al.*, 2012). ST7 strains are also dominant from human isolates and are also responsible for the human outbreaks in China and Hong Kong (Ye *et al.*, 2006, 2008).

In Thailand, most studies have applied MLST to isolates of *S. suis* serotype 2. A summary of *S. suis* molecular studies in Thailand using MLST is presented in Table 5-1. Using *S. suis* serotype 2 isolations from human patients ST1, ST104, ST28, ST101, ST102, ST103, and ST104 were identified. The majority of *S. suis* serotype 2 causing human infection with meningitis and non-meningitis belonged to ST1 (62.4%) and ST 104 (25.5%) (Goyette-Desjardins *et al.*, 2014). ST101-104 is unique in Thailand (Goyette-Desjardins *et al.*, 2014; Kerdsin *et al.*, 2009). Isolates from patients belonged to the clonal complex 1, 27, and 225 (Takamatsu *et al.*, 2008).

S. suis ST16, ST89, ST94, ST104, ST373, ST374, and ST376 were identified from pigs. Pigs isolates were reported of clonal complex 16, 123, and 225.

Table 5-1 *S. suis* molecular study in Thailand by MLST

Reference	Source of sample	Serotype	ST*	Clonal complex**
Takamatsu et al., 2008	Patients	2, 14	1, 25, 28, 101-104	1,27, -
Kerdsin et al., 2011a	Patients	2	1, 104	-
Maneerat et al., 2013	Disease pigs	2	1, 104	1, 225
	Healthy pigs		1, 223, 336	1
	Patients		1, 104	1, 225
Chanto, 2013	Slaughtered pigs	2, ½, 7, 9, 16	16, 89, 94, 104, 373, 374, 375, 376	16, 123, 225, -
Athey *et al.*, 2015	Patients	2	28	-
Hatrongjit *et al.*, 2015	Patients	31	221	221/234
Kerdsin *et al.*, 2015	Patients	9	16	16

*Sequence type from *S. suis* serotype 2 isolates
** Clonal complex provide only from some ST

5.3 Methodology

To gain insights into the population biology of circulating *S. suis* in Chiang Mai we investigated the genetic profile of *S. suis* circulating in pigs in the province.

5.3.1 Samples

S. suis bacteria were obtained from isolates cultured from tonsil swabs collected from backyard pigs in between November 2013 and June 2014, in selected villages in Chiang Mai, northern Thailand (see Chapter 4). Serotyping of all 20 isolates was undertaken at the Veterinary Research and Development Center (Upper Northern Region, Thailand). MLST was performed usng multiplex PCR, at the University of Edinburgh. Of 20 *S. suis* isolates, 14 amplified using MLST.

5.3.2 Capsular Serotyping

DNA was extracted from colonies *S. suis* grown on blood agar at 37ºC. Colonies were dissolved in 1,000 µl distrilled water and centrifuged at 12,000 rpm for 5 minutes. Sediments were dissolved in 100 µl distrilled water, boiled for 10 minutes and chilled. Supernatants were centrifuged at 12,000 rpm for 5 minutes and extracted DNAs were stored at -20 ºC. *S. suis* were serotyped by multiplex PCR. Primers were based on the gene sequences encoding the *16s*, *cps1, cps2, cps7*, *cps9* and *sly* (Table 5-2).

Table 5-2 Primer sequences of PCR

Gene	**Primer sequence (5'-3')**
16s-195(s)	CAG TAT TTA CCG CAT GGT AGA TAT
16as-489(as2)	GTA AGA TAC CGT CAA GTG AGAA
cps 1	GGC GGT CTA GCA GAT GCT CG
	GAG AAC TGT TAG CAA TGAC
cps2	CAA ACG CAA GGA ATT ACG GTA TC
	GAG TAT CTA AAG AAR GCC TAT TG
cps7	GAA TCA ATC CAG TCA GTG TTGG
	CTA ATT CGA TAC GAA GCT AAAC
cps9	GGC TAC ATA TAA TGG AAG CCC
	CCG AAG TAT CTG GGC TAC TG
sly-a3	ATG AGA AAA AGT TCG CAC TTG
sly-a2	ACT CTA TCA CCT CAT CCG C

Multilocus Sequence Typing (MLST)

S. suis, MLST applied 7 housekeeping genes: *aroA* (5-enolpyruvylshikinate 3-phosphate synthase); *cpn60* (60-kDa chaperonin); *dpr* (putative peroxide resistance protein); *gki* (encoding glucose kinase); *mutS* (DNA mismatch repair enzyme); *recA* (homologous recombination factor and *thrA* (aspartiknase /homoserine dehydrogenase). Internal fragments were amplified using the primers shown in **Error! Reference source not found.** using AmpliTaq Gold® 360 Master Mix (Applied Biosystems, California, USA).

PCR reactions were performed in 50 µl volumes using 30 cycles of 95ºC for 1 minute, XºC for 1 minute (where X is 55ºC for *aroA* and *gki*, 52ºC for *cpn* and *thrA*, and 50ºC for *dpr, mutS* and *recA*) and 72ºC for 1 minute. PCR products were visualised on 1.5% agarose gels containing ethidium bromide. PCR products were purified and sequenced by GATC biotech (GATC biotech, Germany). Products were sequenced using the primers applied in the initial PCR, with the exception of *thrA*, (**Error! Reference source not found.**).

5.3.3 Data analysis

The allelic numbers and sequence types (STs) of the isolates were determined by comparing with those in the *S. suis* MLST database (http://pubmlst.ssuis.net). Novel alleles and STs were assigned by submission of the sequence data to the databases. Groups of related genotypes (STs) were analysed with all isolates present in the *S. suis* database by BURST to identify the phylogenetic position of strain and display the overall structure of the population.

Table 5-3 Oligonucleotide primers use for amplification and sequencing in the *S. suis* MLST scheme

Locus	Sequence		Product size (bp)
	Forward (5'-3')	**Reverse (5'-3')**	
dpr	CGTCTTTCAGCCCGCGTCCA	GACCAAGTTCTGCCTGCAGC	434
thrA	GATTCAGAACGTCGCTTTGT	AAGTTTTCATAGAGGTCAGC	523
cpn60	TTGAAAAACGTRACKGCAGGTGC	ACGTTGAAIGTACCACGAATC	466
recA	TATGATGAGTCAGGCCATG	CGCTTAGCATTTTCAGAACC	398
gki	GGAGCCTATAACCTCAACTGG	AAGAACGATGTAGGCAGGATT	480
aroA	TTCCATGTGCTTGAGTCGCTA	ACGTGACCTACCTCCGTTGAC	482
mutS	CGCAGAGCAGATGGAAGATCC	CCCATAGCTGTTTTGGTTTCATC	526

*The primers 5'-AAGAATGGATCATCAACCGT-3' were used for the forward thrA sequencing reaction.

5.4 Results

5.4.1 *S. suis* serotypes

Samples were identified for *S. suis* at the Veterinary Research and Development Center which can identify *S. suis* at serotype level routinely identifying serotypes 1, 2, 7 and 9 that cause infection in humans. Twently isolates from 375 tonsil swab samples were identified as *S. suis* (see Table 5-4). One isolate was identified as serotype 9, and while the remainder were definitively not serotype 1, 2, 7 and 9 it could not be concluded that they were non-typable strains.

Table 5-4 Number of isolate in each cluster with serotype identified.

Cluster	Village	District	Positive sample	No. of isolate	Serotype
1	Wang Tarn	Doi Saket	1	1	n/a
2	Papai	Doi Saket	0	0	n/a
3	Huay Jo	Chom Thong	1	1	**9**
4	Mae Ja	Chiang Dao	2	**3**	n/a
5	Tha Lo	Doi Lo	0	0	n/a
6	Rai Pattana	Doi Lo	2	2	n/a
7	San Kamok	Praw	1	1	n/a
8	Pa Nai	Phrao	1	1	n/a
9	Mae Pang	Phrao	1	1	n/a
10	Huay Khao Lip	Mae Wang	2	2	n/a
11	Nong Tao	Mae Wang	0	0	n/a
12	Mae Sapok	Mae Wang	4	**5**	n/a
13	Pa Ngew	Mae On	0	0	n/a
14	Nam Cham	San Kamphaeng	2	2	n/a
15	Mae Sa Noi	Mae Rim	1	1	n/a
16	Nong Hoi kaw	Mae Rim	0	0	n/a
17	San Pranet	San Sai	0	0	n/a
18	Dong Pa Sang	San Pat Tong	0	0	n/a
19	San Hao	San Pat Tong	0	0	n/a
20	Dong Pa Nhiew	San Pat Tong	0	0	n/a
21	Kong King	Hang Dong	0	0	n/a
22	Pa Hang	Mae Taeng	0	0	n/a
23	Pang Muang	Mae Taeng	0	0	n/a
24	Sop Lerm	Mae Taeng	0	0	n/a
25	Nong Baen	Saraphi	0	0	n/a

5.4.2 Genotyping characterization using Multilocus Sequence Typing *(*MLST*)*

Of 20 *S. suis* isolated from tonsil swabs from backyard pigs in Chiang Mai 14 were genotyped by MLST. Six isolates could not be amplified obtained by MLST, likely due to degradation of DNA. Allele numbers, allelic profiles and sequence types (STs) were logged in the MLST databases for *S. suis* (http://pubmlst.ssuis.org). The individual identification number (ID) of each strain were determined after submission to MLST database.

Twelve STs were determined from the isolates by MLST: ST122, ST318, ST705, ST706, ST707, ST708, ST709, ST710, ST711, ST712, ST713, and ST714 (Table 5-5). Nucleotide sequences from 7 housekeeping genes from twelve of the isolates were found in the database, but the reamining allelic profiles did not match any existing profile in the database and were submitted to http://pubmlst.ssuis.org to assign new ST. Eleven new STs were demonstrated for the first time in Thailand in this study: ST122; T705; ST706; ST707; ST708; ST709; ST710; ST711; ST712; ST713 and ST714. For one sample of serotype 9, ST706 was identified for the first time.

The population genetic diversity of entries in the entire *S. suis* MLST database, including the allelic profile data of the present study, was evaluated with BURST. Result from BURST showed that the allelic profiles from this study were individually unlinked STs that were not single-locus variants or double locus variants of any other STs in the database.

Table 5-5 Allelic profiles and sequence type obtained from backyard pigs in Chiang Mai

ID*	Strain name	Village	Serotype	Clonal complex	STs	Housekeeping gene						
						aroA	cpn60	dpr	gki	mutS	recA	thrA
1570	DK1	SanSai	n/a	singleton	122[a]	53	51	28	7	154	71	33
1571	CD3	MaeJa	n/a	singleton	705[a]	43	58	99	126	109	106	51
1572	CD5	MaeJa	9	singleton	706[a]	77	60	66	7	235	93	28
1573	PR8	Sankmok	n/a	singleton	707[a]	94	71	30	79	67	63	33
1574	PR9	PaNai	n/a	singleton	708[a]	16	58	74	126	159	116	133
1575	PR10	MaePung	n/a	singleton	709[a]	91	100	119	218	19	149	92
1576	MW11	HuiKawleep	n/a	singleton	710[a]	154	58	17	155	81	116	18
1577	MW12	HuiKawleep	n/a	singleton	711[a]	87	34	28	74	135	91	44
1578	MW13	MaeSapok	n/a	singleton	712[a]	168	60	30	74	128	104	79
1579	MW16	MaeSapok	n/a	singleton	713[a]	192	260	166	103	188	157	146
1580	MR20	MaeLanoi	n/a	singleton	714[a]	23	30	7	64	9	3	37
1581	MW17	MaeSapok	n/a	singleton	713[a]	192	260	166	103	188	157	146
1582	SKP18	NamJum	n/a	singleton	318	90	30	11	7	136	77	18
1583	SKP19	NamJum	n/a	singleton	318	90	30	11	7	136	77	18

*ID was assigned by http://pubmlst.org/ssuis/

[a]New sequence types firstly reported in this study

5.5 Discussion

Studies in Thailand have reported serotypes of *S. suis* that are associated with infectious capability, severity, and clinical symptoms. Previous studies identifing *S. suis* from pigs in slaughterhouses in Chiang Mai (Chanto, 2013; Padungtod *et al.*, 2010) used multiplex PCR or serological tests to identify serotypes 2, ½, 7, 9, and 16.

Among all of the serotypes of *S. suis* that have been described, *S. suis* serotype 2 is the most commonly isolated worldwide, causing infection in both humans and pigs (Goyette-Desjardins *et al.*, 2014). Human infection with serotype 2 exhibits clinical signs including meningitis which can lead to hearing loss (Kerdsin *et al.*, 2011a; Navacharoen *et al.*, 2009; Wertheim *et al.*, 2009a). Serotype 2 is also the most frequently isolated serotype from patients in Thailand (Kerdsin *et al.*, 2011a). Other serotypes reported in Thailand include serotype 5, 9, 14, 24, and 31 (Goyette-Desjardins *et al.*, 2014; Hatrongjit *et al.*, 2015; Kerdsin *et al.*, 2009, 2015).

S. suis serotype 2 was not identified in this study. *S. suis* serotype 2 has been previously identified in pigs, at a low prevalence of between 0.3-6% (Chanto, 2013; Pathanasophon *et al.*, 2009, Pathanasophon *et al.*, 2013). This is consistant with a study that revealed raw pork meat, in markets in Hong Kong was contaminated with *S. suis* serotype 2 at low prevelaence (Cheung *et al.*, 2008). Other commonly found serotypes in pigs in Thailand, include serotype 1 and 14 (Pathanasophon *et al.*, 2009, Pathanasophon *et al.*, 2013), neither of which were isolated in this study.

Survival of *S. suis* in human blood and serum is strain dependent. Some *S. suis* strains loose viability in human serum (Khikhuntod *et al.*, 2016). Previous studies demonstrated that *S. suis* serotype 2 can adhere to various types of epithelial cell and

to microvascular endothelial cell of porcine brain tissue (Lalonde *et al.*, 2000; Charland *et al.*, 2000). Studies also found that uncapsulatd serotype 2 and non-typable strains were more adhesive and invasive than encapsulated strains (Benga *et al.*, 2004) and this may explain why some serotypes are more likely to be identified than others.

S. suis serotype 9 was the only serotype isolated from backyard pigs in this study (based on detection of *cps*9H). *S. suis* serotype 9 can cause infection in humans, and Thailand reported its first human cases in 2013 (Kerdsin *et al.*, 2015). The patient was infected after consuming traditional homemade raw pig blood soup, so called "Loo". Although serotype 9 is not commonly found in humans, it is the a common serotype causing disease in pigs in European countries (Allgaier *et al.*, 2001; Tarradas *et al.*, 2004; Wisselink *et al.*, 2000).

The remaining 19 *S. suis* isolates in this study did not belong to serotype 1, 2, 7 and 9. The bacterial isolation and serotype identification in this study was performed at the Veterinary Research and Development Center, Upper Northern Region and with a limited budget, routine work does not screen for all serotypes, and only those most commonly present in humans. *S. suis* serotypes 14 and 16 have been increasing in prevalence in Asian counties (Goyette-Desjardins *et al.*, 2014) and more serotypes should be routinely screened for.

Multiplex PCR is the most common method of identification for *S. suis* worldwide. Non-typable isolates can be identified using this approach if they fail to agglutinate with any typing antisera (Messier, Lacouture and Gottschalk, 2008). Gottschalk *et al*., 2013 demonstrated that 89% of non-typable strains were poorly or non-capsulated. Some non-typable strains have been identified by multiplex PCR

(Okura *et al.*, 2014) and it is recommended that other serotypes should be looked for as routine practice.

S. suis ST1 and ST104 were the most commonly associated STs with human *S. suis* infection in Thailand (Kerdsin *et al.*, 2011a). ST1 is associated with infection in most continents, except for North America where the predominant STs are ST25 and ST28 (Goyette-Desjardins *et al.*, 2014). ST7 is mostly endemic in mainland China, responsible for the1998 and 2005 epidemics (Ye *et al.*, 2006).

In this study MLST was applied to explore the genetic diversity of *S. suis* isolated from backyard pigs. Of 14 isolates obtained by MLST, 13 STs were identified, 12 of which were new STs including ST706 of serotype 9. A previous (human) serotype 9 in Thailand was ascribed to ST16 (Kerdsin *et al.*, 2015). ST318, the only ST that matched to the MLST database (http://pubmlst.ssuis.net) was identified from two isolates from the same village cluster and was an exact match to the allelic profile of isolates from China, which were also obtained from nasal swabs of carrier pigs. The other eleven STs described in this study, are entirely new.

The STs in this study did not group with any existing clonal complex and did not match STs from any other Thai study. The STs identified do not appear to be virulent strains. This study indicates that backyard pigs can carry *S. suis*, and although not carrying the virulent strain, can still a possible reservoir for human infection.

5.6 Summary

In this study, serotyping was performed by multiplex PCR targeting serotype 1, 2, 7 and 9. Sequence typing was undertaken using MLST and sequence data were submitted to MLST database for analysis. In summary the findings show,

1) Of 20 confirmed *S. suis* isolates, one was identified as *S. suis* serotype 9 and 13 isolates were not none of serotypes 1, 2, 7, or 9. Since there was no further identification, the serotypes of the other 13 isolates are unknown.
2) Fourteen isolates were obtained from MLST analysis, of these 12 STs were identified (ST122, ST318, ST705, ST706, ST707, ST708, ST709, ST710, ST711, ST712, ST713, and ST714. ST706).
3) ST318 matched a previously described ST identified from carrier pigs in China. The other eleven were entitely novel STs identified for the first time in this study (ST122, ST705, ST706, ST707, ST708, ST709, ST710, ST711, ST712, ST713, and ST714).
4) For *S. suis* serotype 9 a new ST, ST706 (ID 1572) was identified.
5) The allelic profiles indicated individually unlinked STs that were not single-locus variants or double locus variants of any other STs in the MLST database.

CHAPTER 6-General Discussion

Thailand is endemic for *S. suis* in both humans and pigs. The first case was documented in 1987 (Phuapradit *et al.*, 1987) and *S. suis* has been listed as a notifiable disease in Thailand since 2011 (Wongkamma *et al*., 2014). *S. suis* is part of the normal flora in pigs and has been found across Thailand.

Human *S. suis* infections are commonly acquired when raw meat from infected pigs is consumed. Consumption of raw meat is prevalent in northern regions of Thailand, and most cases are found in these regions. Outbreak investigations show that pigs are still the major source of *S. suis* infection in Thailand. Wongkamma *et. al.*, (2014) found that consumption or close contact to pigs related to 82% of *S. suis* outbreaks. Virulent strains were found in both commercial and backyard pigs, and backyard pigs were the source of infection for some human cases (Khadthasrima *et al.*, 2007a).

This study was undertaken in Chiang Mai province in Northern Thailand, which is highly endemic for *S. suis*. The study examined the epidemiology of *S. suis* for human cases in Chiang Mai, where case reporting was place since 2005, since before became a notifiable disease within the national surveillance system. *S. suis* were examined over a 10-year period from 2005 and 2014 and results showed that *S. suis* has been reported in Chiang Mai every year with an incidence rate of between 1.21 to 49.69 per 1,000,000 population. This is 6.5 times higher than for the rest of Thailand. The average fatality rate was 10.12%.

The health and economic burdens of human *S. suis* infection were explored in Chiang Mai. Health burden, presented as disability adjusted life year (DALY) using 2013 as baseline, amounted to 129.81 or 7.41 per 100,000 population. A high burden

was observed in the 15-44 age group. The economic burden was estimated based on cases from 2013 and 2014 using recorded data and interviews with cases. On average, a case of *S. suis* infection cost the national health security system 37,955 baht (£759). The cost to the patient in out of pocket expenses was on an average 5,198 baht (£104).

Most *S. suis* studies in Thailand have focused on identifying *S. suis* in commercial pigs. The virulent strain of *S. suis*, serotype 2, was isolated from backyard pigs in a previous study in Chiang Mai (Padungtod *et al.*, 2010). Outbreak investigations in this region confirmed that backyard pigs are also a source of infection (Khadthasrima *et al.*, 2007a).

In this study, *S. suis* was isolated from backyard pigs in Chiang Mai. The prevalence was 4.8% (95%CI=2.2-7.4), lower than that previously reported in slaughtered pigs in Chiang Mai (Chanto, 2013; Lakkitjaroen *et al.*, 2009). The major risk factor for infection in backyard pigs was overcrowding. Strains isolated from the backyard study were characterized by serotyping and multilocus sequence typing. The only *S. suis* serotype identified in this study belonged to serotype 9, which is the human infective strain. Using MLST twelve unique sequence types were identified and theses profiles were submitted to MLST database at http://pubmlst.ssuis.org.

While management practices can be introduced to reduce transmission of *S. suis* in pig farms (Gottschalk, 2010), there is no measure to prevent healthy carrier pigs getting into the food chain. The only control measure to prevent human infection available is public education providing advice on how to cook meat correctly. A risk communication model has been implemented in some villages in Thailand that had experienced previous large outbreaks (Khamlar *et al.*, 2013). However, traditional raw

dishes are still popular and it is difficult to change this cultural practice. *S. suis* infection has continually been reported from raw meat consumption, suggesting that attempts to inform the people of the danger of eating raw meet have not yet met with success. The public education campaign needs to be re-evaluated.

6.1 Current situation of *S. suis*

S. suis is one of the five most important zoonosis in Thailand, the zoonoses being rabies, avian influenza, leptospirosis and brucellosis (Lakkitjaroen *et al.*, 2008). *S. suis* has the second highest numbers of cases among these five zoonoses. In 2014, 359 cases of *S. suis* were reported with morbidity and mortality rates of 0.55 and 0.03 per 100,000 population respectively. The highest incidence of *S. suis* was in the northern region with a morbidity rate 2.09 per 100,000 populations, after leptospirosis with 2,171 cases and morbidity and mortality rates of 3.30 and 0.08 per 100,000 population. There were 6 cases of rabies reported and no cases of avian influenza in 2014 (http://boe.moph.go.th).

6.2 Factors influencing *S. suis*

From previous outbreak investigations and studies, consumption of raw or semi-cooked pork is a significant risk factor for *S. suis* infection (Ho *et al.*, 2011; Khadthasima *et al.*, 2007). The cultural eating habits of people across Asia influence the occurrence of foodborne diseases including opisthorchis, trichinellosis and *S. suis* (Andrews *et al.*, 2008; Fongcom *et al.*, 2001; Kongkaew, 2011; Kunnalok, 1996; Mai *et al.*, 2008).

Although, the practice of eating raw meat is the main risk factor for *S. suis* infection in Thailand, other behaviors can increase the risk of infection and disease

severity, including regular alcohol consumption. Alcohol consumption leads to liver damage and is a predisposing factor for *S. suis* infection, facilitating translocation of *S. suis* from the intestine to the circulatory system and the brain (Nakayama *et al.*, 2013). Immunosuppressive conditions can also predispose individuals to *S. suis* infection (Gómez-Zorrilla *et al.*, 2014). Other diseases including heart disease, diabetes and cancer are also risk factors for human *S. suis* infection (Voutsadakis, 2006; Wangkaew *et al.*, 2006; Wertheim *et al.*, 2009a).

Secondary risks for *S. suis* infection include poor protection when handling pork meat, especially having cuts or sores on hands, affecting people working and preparing meat, including caterers, housewives, and butchers. Buying meat from unreliable sources increases the risk of infection (Ho *et al.*, 2011; Padungtod *et al.*, 2010). Workers in pig farms should also wear personal protective clothing when handling with pigs to reduce chance of getting infection.

This study found that backyard pigs are maintained in communities in Chiang Mai, mainly as a source of secondary income. Although individual holdings have established different forms of pig housing (concrete floor, dirt-floor, traditional pit house and elevated wood floor), the management practices and and characteristics of the holdings were similar. Overcrowding was the only identifiable risk factor influencing *S. suis* infection in this study which corresponded to a previous study (Padungtod *et al.*, 2010). Overcrowding, together with poor ventilation increases the opportunity for bacterial transmission in the herd (Gottschalk, 2010).

In this study, most backyard pig farms were managing by housewives. The lower incidence of *S. suis* in females suggests that handling living pigs is a lower risk

for infection than consumption of raw meat. There are only few cases of reported infection from close contact with pigs in Thailand (Wongkamma *et al*., 2014). This is similar to the situation in Vietman where most patients are infected by eating raw pork (Ho *et al.*, 2011), but in sharp contrast to the situation in industrialised countries and in China, where *S. suis* infection mosty arises through close contact or handling of pigs (Huong *et al.*, 2014).

Backyard pig rearing poses a lower risk to humans for *S. suis* than commercial pig rearing. In this study, there was a lower prevalence of *S. suis* in backyard pigs than has been observed in the commercial pig production system in Chiang Mai. The number of commercial pigs in Chiang Mai is 5 times higher than for backyard pigs (www.region5.dld.go.th) and more meat from commercial production is going into the food chain.

Commercial farms and slaughterhouses are regulated and this should reduce risk. However, since *S. suis* is part of the normal flora of pigs and infection can be silent there is a risk. Thailand imports 200-300 pigs from breeders in Europe and the United States each year. Although, not high, importation may introduce novel virulent strains or sequence types and *S. suis* surveillance should be focussed on commercial pig units.

6.3 *S. suis* control measures

6.3.1 Control measure in human

The main risk factor for acquiring *S. suis* in human in Thailand (around 68%) is from consumption of raw or semi-cooked pork and its products (Wongkamma *et al*., 2014). This is followed by close contact to pigs or pig product (26%), and 3% each for

consumption of buffalo meat and undetermined etiologies. Since *S. suis* is difficult to eliminate from pigs since it is part of the normal flora and can be found in healthy pigs, the rationale for control of *S. suis* should focus on prevention of human infection. Health education is required to avoid people consuming raw pork.

Human *S. suis* usually occurs in groups of individuals (mostly men) who gather together, after work or at festivals, drinking and eating raw traditional dishes (Khadthasima *et al.*, 2007; Wongkamma *et al*., 2014). Outbreaks are sporadic and tend to cluster during the months that have important festivals and gathering events.

There are strong cultural beliefs around raw meat consumption. Some people believe that raw meat is more nutritious than cooked meat, and that it is healthier to eat raw meat. Some believe that preparing raw meat with lime or drinking alcohol with raw meat, kills any bacteria in the meat (Chokvanichpong *et al*., 2009; Khamlar *et al.*, 2013). Others consider that since the previous generations ate raw pork dishes, and were healthy, that the practice is safe. Some believe that eating a small amount of raw food only on special occasions lessens the risk of infection (Kongkaew, 2011).

The Ministry of Public Health and local health authorities should implement campaigns to reduce the practice of eating raw meat. It is difficult to change cultural practices and appropriate messaging needs to be deployed. Most interviewees in this study knew they should not eat raw or lightly cooked meat, but, more than 95% of still ate raw meat. Even if adults within the community are reluctant to change their habit, it may be possible to influence behaviour in children if they are given appropriate knowledge (Morgan, 2014). Providing knowledge to children at school may help change behavior that could lead to a decrease in risky behavior in the future.

Communities also need to know how to select quality pork and to be advised to buy pork from certified slaughterhouses. Healthy pork should appear, reddish pink to purplish red, consumers should be advised to avoid dark red or meat congested with blood as it this may have come from septicaemic animals (FAO, n.d.). Persons preparing pork and other meat should wash their hands thoroughly with detergent after handling raw pork and any viscera, especially if they have a wound/lesion.

Although, certified farms and slaughterhouses do not guarantee *S. suis* free meat, pigs from these farms and slaughtered in certified slaughterhouses are in good health, healthy pigs will be less at risk of spreading *S. suis*. They are also at less risk for PRRS, swine influenza, and bacterial diseases that can promote overgrowth of *S. suis*, decreasing the risk of *S. suis* contaminated meat reaching the consumer (Galina *et al.*, 1994; Thanawongnuwech *et al.*, 2000; Barre, 2015). Certified slaughterhouses also employ good slaughterhouse management practices. These include, good lairage areas, proper animal inspection to avoid sick pigs entering the unit, as well as meat inspection to lower the risk of meat contaminated with *S. suis* entering the food chain (Neungmek and Pathanasophon, 2011).

S. suis can infect individuals having close contacts with pigs or pig carcasses through wounds or abrasion on the skin, especially the hands and arms. In this study, none of the farmers were aware that *S. suis* could be transmitted in this manner. Slaughterhouse workers may also be unaware of this risk. The Ministry of Health together with the Department of Livestock Development should provide knowledge to farmers and workers in slaughterhouses to avoid handling pigs or carcasses with bare hands and recommend showering after handling pigs. Any existing wounds should be appropriately covered before handling pigs.

The consequences of *S. suis* infection are serious in humans. Meningitis can be fatal. Prompt diagnosis and medical care are essential to prevent serious and permanent injury or death. Many cases of *S. suis* infection were not confirmed by microbiological culture (Hmonpangtiam and Chuknam, 2011). In this study, in Chiang Mai province, only the provincial hospital could make a confirmed diagnosis of *S. suis.* None of the district hospitals could make a diagnosis of *S. suis*. Treatments were based on patient clinical signs and case history only. The lack of laboratory confirmation will impact on underreporting of *S. suis*. Strengthening laboratory networks should be a priority for development of *S. suis* surveillance and control in Chiang Mai and more broadly across Thailand.

Tis study also indicated that the patient needed to be provided with more information of *S. suis* before they are discharged. Although patients knew they were sick and needed hospitalised, there was a lack of awareness about their disease and impacts. Healthcare personnel should provide information about how the patient acquitted the infection and about living with the consequences of *S. suis* infection before discharge.

6.3.2 Control measure in pigs

S. suis can cause severe loss to the pig industry, especially in the case of co-infection with PRRS (Hoa *et al.*, 2013; Thanawongnuwech *et al.*, 2000). On farm control of *S. suis* on farms is focused on farm management and control of any predisposing infections, including; PRRS, Aujesky's disease, mycoplasmosis and swine influenza. Even if *S. suis* is not causing disease on the farm the bacteria are still present as normal flora in healthy pigs. In this study, *S. suis* serotype 9, associated

with human infection, was identified in a backyard pig in Chiang Mai. A human *S. suis* serotype 9 infection was reported in Thailand in 2013 (Kerdsin *et al*., 2015). The ST of the outbreak strain was of ST16 which is a different ST from that identified in the pig in this study. Rattanamaneekorn and Oopakornrat (2014) have recently reported three *S. suis* serotypes for human infection in backyard pigs. These studies suggest that backyard pigs may be a reservoir of human infection in Chiang Mai. None of the backyard pig holders interviewed in this study were aware of *S. suis*.

Overcrowding is a main risk factors for causing of *S. suis* transmission on pig farms (Dee *et al.*, 1993; Gottschalk, 2010; Quinn *et al.*, 2011). This study showed that pigs living in small spaces (less than 1.2 m^2), was a risk factor of *S. suis* infection in pigs. The Department of Livestock Development has recommended that pigs should have 1.2-1.5 m^2 of space to lessen stress from overcrowding (Bureau of Livestock standards and certification, 1999). Simple adjustments to the pig housing management systems can reduce the opportunity for *S. suis* to spread in farms (Dekker *et al.*, 2013)., Overcrowding can lead to overheating, increased stress, and higher ammonia and dust levels that impact negatively on the defenses of the respiratory tract and can cause pigs to become susceptible to respiratory diseases including PRRS, *Mycoplasma hyopneumoniae*, and swine influenza (Brockmeier *et al*., 2002).

Another method to prevent *S. suis* spread is to practice depopulation and repopulation with cleaning and disinfection.

Unlike for *S. suis* human outbreaks, an outbreak of *S. suis* in pigs does not need to be reported. No *S. suis* infection has been diagnosed by the Veterinary Research and Development Center, over the past 10 years in Thailand. Although several outbreaks

of PRRS have been reported in Chiang Mai, *S. suis* was never diagnosed. Since *S. suis* has often been reported from human and slaughtered pigs, veterinarians should be aware of this disease and able to identify infected pigs, on the farm.

6.4 Prospects for *S. suis* control

Of over 1,500 human cases worldwide *S. suis* reported between 1968-2012, most arose from China and Southeast Asia (Vietnam and Thailand). Sporadic cases have been reported across Europe where *S. suis* is considered an occupational disease, occurring in people working in close contact with pig and pork products (Huong *et al.*, 2014). Although *S. suis* cases from European countries constitute around 10% of total *S. suis* cases globally, only the United Kingdom (in 1983) and France (in 1995) have listed *S. suis* infection in humans as an industrial disease (Gottschalk, 2004), and legislation and regulations are in place to control the disease (Goyette-Desjardins *et al.*, 2014). In UK, *S. suis* is reportable according under the Reporting of Injuries, Diseases and Dangerous Occurrences Regulation of 1995 (www.legislation.gov.uk).

Preventive strategies have been developed in *S. suis* endemic countries. In Hong Kong, where *S. suis* is mostly related to occupational exposure, *S. suis* has, since 2005, been a notifiable occupational disease under the Occupational Safety and Health Ordinance. The Occupational Safety and Health Council of Hong Kong has enacted legal requirements and schemes to promote the safety of workers (http://oshc.org.hk), including, legislation on "occupational safety and health ordinance" and messaging for "prevention of *Streptococcus suis* infection".

Most human cases of *S. suis* (70%) in Vietnam have been caused by consumption of raw pork or pork products such as "blood pudding". The remaining

cases were infected while slaughtering pigs (Harriman, 2015). A study to develop national *S. suis* guidelines was conducted in 2007 (Horby *et al.*, 2010) and in 2009, the Ministry of Health issued national guidelines for *S. suis* (Ministry of Health Decision 3065/QD-BYT). Guidelines were sent to all hospitals in Vietnam and include guidelines for the diagnosis and treatment of *S. suis*. Guidelines for surveillance and control of communicable diseases were updated in 2007, and included a section dedicated to *S. Suis*. Guidelines include advice to not slaughter or consume sick pigs, to cover wounds and wear protective equipment when slaughtering of pigs, and not to consume undercooked pork (Horby *et al.*, 2010).

Thailand, has advanced control for *S. suis*. *S. suis* is listed as a notifiable disease and guidelines for *S. suis* identification and case management have been distributed to all hospitals. Many campaigns have been launched to prevent *S. suis* infections in humans but despite these *S. suis* human cases are consistently reported each year. As can be seen in this study, it is difficult to change traditional practice in the community that involve the consumption of raw pork and meat products. Furthermore, as seen in this study, since individuals are covered under the Health insurance scheme to access free treatment, they are not concerned about the large costs of treatment, although individual out of pockets are a significant household burden. It is essential to provide education to raise awareness of *S. suis*; on the severity of the disease, highlighting the economic impacts on the household and flagging the risk factors for infection including consumption of alcohol and other health conditions that potentiate infection.

To control *S. suis*, potential risks should be minimized through animal health and husbandry control measures and improved slaughter processes. Animal for

slaughter should be inspected for sign of clinical illness. Meat inspection procedures should be managed by trained personnel to identify any signs of disease in the carcass.

Given that *S. suis* can be carried by healthy pigs, and is difficult to detect, either at farm or slaughterhouse, to minimize contamination, the principles of food hygiene must to applied throughout the entire food chain, from production, slaughter, meat processing and food preparation. Around 50,000-60,000 pigs are slaughtered daily in Thailand for domestic consumption. The high numbers of pigs slaughtered leads to poor animal inspection both before and after slaughter and can result in unsafe meat entering the food chain. By contrast, most poultry abattoirs meet international standards since most of the poultry is for export.

According to Animal Slaughter and Retailing Act of 1992 and the Ministerial Regulation on Rules Methods and Condition for Abattoir Establishment (2012), meat from animal which is not slaughtered in a standard abattoir is not permitted to be sold in the market. The Department of Livestock Development has a duty to develop abattoirs that meet the national standard. To Department of Livestock Development has provided standard abattoir construction plans, and provide officer to give advice for slaughterhouse improvement to meet these standards, as well providing an investment fund for abattoir operators. The aim is to have standard abattoir practices to serve every municipality and all abattoirs need to register with the Department of Livestock Development. Every abattoir must have a meat inspector who is required to have Degree of Veterinary Medicine, or Animal Husbandry, or Veterinary technical qualification, trained by the Animal Husbandry Association of Thailand. Thailand has 1,872 registered abattoirs (http://dld.go.th) that need to have at least one meat inspector but Thailand does not have enough meat inspectors. To temporarily overcome this

problem, the Department of Livestock Development allows Provincial Veterinary Officers and District Veterinary Officer to temporarily work as meat inspector in abattoirs that cannot find a permanent meat inspector.

6.5 Future research

Human behavior is an important risk factor for *S. suis* infection. From this study only the families of patients that have been severely affected by *S. suis* are likely to stop their traditional practice of consumption of raw meat. Future work should examine the reasons that people prefer to consume raw meat and explore how such behavior could be modified. Although traditional dishes are preferred consumed raw, they can be prepared cooked. The influence of ethnicity on risk for *S. suis* infection should also be explored further to determine how to target health messaging. Most of *S. suis* cases were reported in Thai people rather than minority ethnic people.

There is a lack of data on *S. suis* in live pigs. A large scale molecular epidemiological study of *S. suis* to determine the relationship between *S. suis* in pigs and those obtained from human patients should be undertaken. To date *S. suis* has never been reported by veterinarians from live pigs in the northern region of Thailand according to the record of the Veterinary Research and Development Center (Upper northern region). Previous studies of slaughtered pigs in mixed commercial and backyard system in Chiang Mai identified *S. suis* of serotype 2 (Chanto, 2013; Wongsawan *et al.*, 2006; Padungtod *et al.*, 2010). While in this study of live backyard pigs, serotype 2 was not identified, the very high level of diversity of circulating strains of *S. suis* identified in backyard pigs, should be further explored. Goyette-Desjardins *et al.* (2014) comented that "though the MLST data currently available for *S. suis*

strains isolated from both diseased pigs and human cases of infection come from different countries throughout the world, there remains a long way to go before a complete picture of the current situation of *S. suis* can be obtained". Future research should investigate *S. suis* from the farm to the slaughterhouse determine the prevalence of *S. suis* at all parts of the food chain.

Poor management can cause stress in pigs and the emergence of *S. suis* infection (Gottschalk, 2010). *S. suis* is highly prevalent in immunosuppressed pigs co-infected with PRRS, swine influenza, and *Mycoplasma hyorhinis* (Thanawongnuwech *et al.*, 2000). An investigation to compare the prevalence of *S. suis* prevalence in immunosuppressed herds and herds that free from immunosuppressive diseases should a priority for future work.

References

Abramson, J.H. (2011) *WINPEPI update: computer programs for epidemiologist, and their teaching potential*. Epidemiologic Prospective and Innovations. 8(1), doi: 10.1186/1742-5573-8-1

Allgaier, A., Goethe, R., Wisselink, H.J., Smith, H.E. and Valentin-Weigand, P. (2001) Relatedness of *Streptococcus suis* isolates of various serotypes and clinical backgrounds as evaluated by macrorestriction analysis and expression of potential virulence traits. *Journal of Clinical Microbiology*. 39(2), 445-453.

Amass, S.F., Clark, L.K., Knox, K., Wu, C.C. and Hill, M.A. (1996) *Streptococcus suis* colonization of piglets during parturition. *Swine Health Production*. 4(6), 269-272.

Amass, S.F., Clark, L.K. and Wu, C.C. (1995) Source and timing of Streptococcus. *Swine Health and Production*. 3(5), 189.

Andrews, R.H., Sithithaworn, P. and Petney, T.N. (2008) *Opisthorchis viverrini*: an underestimated parasite in world health. *Trends in Parasitology*. 24(11), 497-501.

Anon (2008) *National Food Committee Act (2008)* [online]. Available from: http://www.acfs.go.th/km/download/act_food_committee_2551.pdf [Accessed 9 December 2015].

Anon (2014a) *Project and Progress Review: Agricultural Quality Development Project, Department of Livestock Development* [online] pp. 27. Available from: http://www.opsmoac.go.th/download/iocd57/Burana1/a-1.pdf [Accessed 9 December 2015].

Anon (2014b) *Thailand Livestock Census 2014* [online]. Available from: http://ict.dld.go.th/th2/images/stories/stat_web/yearly/2557/book2557/05.pdf [Accessed 8 December 2015].

Anon (2014c) Truth of Thai health system *ThaiPublica* [online]. Available from: http://thaipublica.org/2014/07/narong-sahametapat-health-care-system-in-thailand/ [Accessed 22 January 2016].

Anon (2015a) *Agriculture, value added (% of GDP) | Data | Table*. Available from: http://data.worldbank.org/indicator/NV.AGR.TOTL.ZS [Accessed 8 December 2015].

Anon (2015b) *Thailand Population Census*. Available from: http://service.nso.go.th/nso/web/statseries/statseries01.html [Accessed 8 December 2015].

Anon (2015c) *Volume and Value of livestock and product export since 2005-2015*. Available from: http://planning.dld.go.th/th/index. [Accessed 8 December 2015].

Anon (n.d.) *WHO | Public health surveillance*. Available from: http://www.who.int/topics/public_health_surveillance/en/ [Accessed 9 December 2015].

Arai, S., Tohya, M., Yamada, R., Osawa, R., Nomoto, R., Kawamura, Y. and Sekizaki, T. (2015) Development of loop-mediated isothermal amplification to detect *Streptococcus suis* and its application to retail pork meat in Japan. *International Journal of Food Microbiology*. 208, 35-42.

Areechokchai, D. (2014) *Revised Coding of Diseases in National Epidemiological Surveillance, 2012*. 4 (7), Available from: http://www.cpdho.go.th/attachments/article/427/edit_code506_DNAS.pdf [Accessed 9 December 2015].

Arends, J.P. and Zanen, H.C. (1988) Meningitis caused by *Streptococcus suis* in humans. *Reviews of Infectious Diseases*. 10(1), 131-137.

Aschalew, Z.B., Yogesh, C., Jonathan, E., John, T. and Sagar, M.G. (2014) Development of a loop-mediated isothermal amplification assay for rapid detection of *Streptococcus suis* serotype 2. *African Journal of Microbiology Research*. 8 (19), 1955-1959. doi:10.5897/AJMR2013.6281.

Aschengrau, A. and Seage, G.R. (2014) Essentials of epidemiology in public health. 3rd ed. Burlington, MA: Jones & Bartlett Learning. pp. 526.

Asma, I., Sim, B.L.H., Brent, R.D., Johari, S. and Yvonne Lim, A.L. (2015) Molecular epidemiology of Cryptosporidium in HIV/AIDS patients in Malaysia. *Tropical Biomedicine*. 32(2), 310-322.

Athey, T.B.T., Auger, J.-P., Teatero, S., Dumesnil, A., Takamatsu, D., Wasserscheid, J., Dewar, K., Gottschalk, M. and Fittipaldi, N. (2015) Complex Population Structure and Virulence Differences among Serotype 2 S*treptococcus suis* Strains Belonging to Sequence Type 28. *PLoS One*. 10 (9): e0137760. doi: 10.1371/journal.pone.0137760

Baele, M., Chiers, K., Devriese, L.A., Smith, H.E., Wisselink, H.J., Vaneechoutte, M. and Haesebrouck, F. (2001) The gram-positive tonsillar and nasal flora of piglets before and after weaning. *Journal of Applied Microbiology*. 91(6), 997-1003.

Bailey, L. (2005) Introduction to epidemiology [online]. Maidenhead: Open University Press. [Accessed 20 February 2014].

Barre, A. (2015) The relationship between *Streptococcus suis* and Haemophilus parasuis, two important residents of the tonsils of the soft palate in swine. [online]. Available from: https://atrium.lib.uoguelph.ca/xmlui/handle/10214/9204 [Accessed 10 January 2017].

Baums, C.G., Kock, C., Beineke, A., Bennecke, K., Goethe, R., Schroder, C., Waldmann, K.-H. and Valentin-Weigand, P. (2009) *Streptococcus suis* Bacterin and Subunit Vaccine Immunogenicities and Protective Efficacies against Serotypes 2 and 9. *Clinical and Vaccine Immunology*. 16(2), 200-208.

Beef cattle strategy committee (2013) Beef cattle strategy [online]. Available from: http://www.dld.go.th/th/images/stories/news/Strategy/55-59%20strategy_beef.pdf [Accessed 21 January 2016].

Benga, L., Goethe, R., Rohde, M. and Valentin-Weigand, P. (2004) Non-encapsulated strains reveal novel insights in invasion and survival of Streptococcus suis in epithelial cells. *Cellular Microbiology*. 6(9), 867-881.

Bintvihok, A. and Davitiyananda, D. (2003) Aflatoxins and their metabolites residues in swine tissues from 7 regions of Bangkok and near-by, Thailand. 17(1), 11-22.

Blume, V., Luque, I., Vela, A.I., Borge, C., Maldonado, A., Domínguez, L., Tarradas, C. and Fernández-Garayzábal, J.F. (2009) Genetic and virulence-phenotype characterization of serotypes 2 and 9 of S*treptococcus suis* swine isolates. *International Microbiology: The Official Journal of the Spanish Society for Microbiology*. 12(3), 161-166.

Boonchoo, P. (2012) Implementation of International Health Regulation *(*2005*)*. [online]. Thammasat University. Available from: http://digi.library.tu.ac.th/thesis/la/1908/title-biography.pdf [Accessed 9 December 2015].

Bosch, A.A.T.M., Biesbroek, G., Trzcinski, K., Sanders, E.A.M. and Bogaert, D. (2013) Viral and Bacterial Interactions in the Upper Respiratory Tract. *PLoS Pathogens*. 9(1):e1003057. doi: 10.1371/journal.ppat.1003057.

Boye, M., Feenstra, A.A., Tegtmeier, C., Andresen, L.O., Rasmussen, S.R. and Bille-Hansen, V. (2000) Detection of *Streptococcus suis* by in Situ Hybridization, Indirect Immunofluorescence, and Peroxidase-Antiperoxidase Assays in Formalin-Fixed, Paraffin-Embedded Tissue Sections from Pigs. *Journal of Veterinary Diagnostic Investigation*. 12(3), 224-232.

Brockmeier, S.L., Halbur, P.G. and Thacker, E.L. (2002) Porcine Respiratory Disease Complex [online]. ASM Press. [Accessed 12 February 2017].

Broeck, J. van den and Brestoff, J.R. (2013) Epidemiology*:* principles and practical guidelines. Dordrecht; New York: Spinger. pp.621.

Bundhamcharoen, K., Odton, P., Phulkerd, S. and Tangcharoensathien, V. (2011) Burden of disease in Thailand: changes in health gap between 1999 and 2004. *BMC Public Health*. 11, 53.

Bureau of Epidemiology (2006) Annual Epidemiolgical Surveillance Report 2006 [online]. Available from: http://www.boe.moph.go.th/Annual/Annual49/Executive%20summary.html [Accessed 9 December 2015].

Bureau of Epidemiology (2013) Annual Epidemiological Surveillance Report 2013 [online]. Available from: http://www.boe.moph.go.th/Annual/AESR2013/index.php [Accessed 9 December 2015].

Bureau of Epidemiology (n.d.) Bureau of Epidemiology, Thailand. Available from: http://203.157.15.110/boe/home.php [Accessed 9 December 2015].

Bureau of general communicable diseases (2007) Guidelines to prevention and control *Streptococcus suis*. Bangkok: The war veterans' organization of Thailand under royal patronage of His Majesty the King. pp. 176.

Bureau of Livestock standards and certification (1999) Standard pig farming of Thailand 1999. Available from: http://certify.dld.go.th/certify/index.php/th/ [Accessed 9 December 2015]

Bureau of the Budget (2015) *Thailand Budget 2015* [online]. Available from: http://www.mua.go.th/users/budget/doc/budget_58.pdf [Accessed 22 January 2016].

Byra, C., Gadbois, P., Cox, W.R., Gottschalk, M., Farzan, V., Bauer, S.A. and Wilson, J.B. (2011) Decreased mortality of weaned pigs with *Streptococcus suis* with the use of in-water potassium penicillin G. *The Canadian Veterinary Journal*. 52(3), 272-276.

Callejo, R., Prieto, M., Salamone, F., Auger, J.-P., Goyette-Desjardins, G. and Gottschalk, M. (2014) Atypical *Streptococcus suis* in Man, Argentina, 2013. *Emerging Infectious Diseases*. 20(3), 500-502.

Callens, B.F., Haesebrouck, F., Maes, D., Butaye, P., Dewulf, J. and Boyen, F. (2013) Clinical resistance and decreased susceptibility in *Streptococcus suis* isolates

from clinically healthy fattening pigs. *Microbial Drug Resistance (Larchmont, N.Y.)*. 19(2), 146-151.

del Campo Sepúlveda, E.M., Altman, E., Kobisch, M., D'Allaire, S. and Gottschalk, M. (1996) Detection of antibodies against *Streptococcus suis* capsular type 2 using a purified capsular polysaccharide antigen-based indirect ELISA. *Veterinary Microbiology*. 52(1-2), 113-125.

Carricoteam, J.A., Sabat, A.J. and Romirez, A.W. (2013) Bioinformatics in bacterial molecular epidemiology and public health*:* databases, tools and the next-generation sequencing revolution. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20382 [Accessed 8 March 2016].

CDC (2006) Principles of Epidemiology in Public Health Practice*:* An Introduction to Applied Epidemiology and Biostatistics [online]. 3rd edition. Atlanta, USA: U.S. Department of Health and Human Services. [Accessed 9 December 2015].

Chang, B., Wada, A., Ikebe, T., Ohnishi, M., Mita, K., Endo, M., Matsuo, H., Asatuma, Y., Kuramoto, S., Sekiguchi, H., Yamazaki, M., Yoshikawa, H., Watabe, N., Yamada, H., et al. (2006) Characteristics of *Streptococcus suis* isolated from patients in Japan. *Japanese Journal of Infectious Diseases*. 59(6), 397-399.

Chanto, W. (2013) Prevalence and genetic diversity of *Streptococcus suis* in healthy finishing pigs in Chiang Mai and Lamphun, Thailand. Thesis (Master), Chiang Mai University.

Chao, Y., Marks, L.R., Pettigrew, M.M. and Hakansson, A.P. (2015) *Streptococcus pneumoniae* biofilm formation and dispersion during colonization and disease. *Frontiers in Cellular and Infection Microbiology* [online]. 4. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4292784/ [Accessed 16 February 2017].

Charland, N., Nizet, V., Rubens, C.E., Kim, K.S., Lacouture, S. and Gottschalk, M. (2000) *Streptococcus suis* serotype 2 interactions with human brain microvascular endothelial cells. *Infection and Immunity*. 68(2), 637-643.

Charoensook, R., Knorr, C., Brenig, B. and Gatphayak, K. (2013) Thai pigs and cattle production, genetic diversity of livestock and strategies for preserving animal genetic resources. 7(1), 113-132.

Chau, P.Y., Huang, C.Y. and Kay, R. (1983) *Streptococcus suis* meningitis. An important underdiagnosed disease in Hong Kong. *The Medical Journal of Australia*. 1(9), 414-417.

Chen, B., Zhang, A., Li, R., Mu, X., He, H., Chen, H. and Jin, M. (2010) Evaluation of the protective efficacy of a newly identified immunogenic protein, HP0272, of *Streptococcus suis*. *FEMS Microbiology Letters*. 307(1), 12-18.

Chen, L., Song, Y., Wei, Z., He, H., Zhang, A. and Jin, M. (2012) Antimicrobial Susceptibility, Tetracycline and Erythromycin Resistance Genes, and Multilocus Sequence Typing of *Streptococcus suis* Isolates from Diseased Pigs in China. *The Journal of Veterinary Medical Science / the Japanese Society of Veterinary Science*. 75(5):583-7

Cheung, P.-Y., Lo, K.L., Cheung, T.T., Yeung, W.H., Leung, P.H. and Kam, K.M. (2008) *Streptococcus suis* in retail markets: how prevalent is it in raw pork? *International Journal of Food Microbiology*. 127(3), 316-320.

Choi, B.C.K. and Choi, B.C.K. (2012) The Past, Present, and Future of Public Health Surveillance. *Scientifica (Cairo),* 2012: 875253. doi: 10.6064/2012/875253.

Chokvanichpong, V., Sarakhan, A. and Sangrajrang, S. (2009) Study of behavoirs and attitudes related to eating raw freshwater fish and cholangiocarcinoma. *Thai Cancer Journal*. 29(4), 162-175.

Choprapawon, C., Porapakkham, Y., Sablon, O., Panjajaru, R. and Jhantharatat, B. (2005) Thailand's national death registration reform: verifying the causes of death between July 1997 and December 1999. *Asia-Pacific Journal of Public Health /Asia-Pacific Academic Consortium for Public Health*. 17(2),110-116.

Chorba, T.L., Berkelman, R.L., Safford, S.K., Gibbs, N.P. and Hull, H.F. (1989) Mandatory Reporting of Infectious Diseases by Clinicians. 262(21), 3018-3026.

Chotmongkol, V., Janma, J. and Kawamatawong, T. (1999) *Streptococcus suis* meningitis: report of a case. *Journal of the Medical Association of Thailand: Chotmaihet thangphaet*. 82(9), 922-924.

Chuxnum, T. (2009) *Streptococcussuis*. Available from: http://www.sbo.moph.go.th/moph/develop/rabad/Streptococcussuis.pdf [Accessed 12 August 2014].

Clifton-Hadley, F.A., Alexander, T.J., Upton, I. and Duffus, W.P. (1984) Further studies on the subclinical carrier state of *Streptococcus suis* type 2 in pigs. *The Veterinary Record*. 114(21), 513-518.

Clifton-Hadley, F.A. and Enright, M.R. (1984) Factors affecting the survival of Streptococcus suis type 2. *The Veterinary Record*. 114(24), 584-586.

Clifton-Hadley, F.A., Enright, M.R. and Alexander, T.J. (1986) Survival of *Streptococcus suis* type 2 in pig carcases. *The Veterinary Record*. 118(10), 338-341.

Conteh, L., Engels, T. and Molyneux, D.H. (2010) Socioeconomic aspects of neglected tropical diseases. *Lancet*. 375 (9710), 239-247.

Dang, Y., Lachance, C., Wang, Y., Gagnon, C.A., Savard, C., Segura, M., Grenier, D. and Gottschalk, M. (2014) Transcriptional approach to study porcine tracheal epithelial cells individually or dually infected with swine influenza virus and *Streptococcus suis*. *BMC Veterinary Research*. 10(1), 86. doi: 10.1186/1746-6148-10-86.

D'Argenio, V. and Salvatore, F. (2015) The role of the gut microbiome in the healthy adult status. *Clinica Chimica Acta*. 451, Part A, 97-102.

Declich, S. and Carter, A.O. (1994) Public health surveillance: historical origins, methods and evaluation. *Bulletin of the World Health Organization*. 72(2), 285-304.

Dee, S.A., Carlson, A.R., Winkelman, N.L. and Corey, M.M. (1993) Effect of management practices on the *Streptococcus suis* carrier rate in nursery swine. *Journal of the American Veterinary Medical Association*. 203(2), 295-299.

Dee, S.A. and Corey, M.M. (1993) The survival of *Streptococcus suis* on farm and veterinary equipment. *Swine Health and Production*. 1(1), 17-20.

Dekker, N., Bouma, A., Daemen, I., Klinkenberg, D., Leengoed, L. van, Wagenaar, J.A. and Stegeman, A. (2013) Effect of Spatial Separation of Pigs on Spread of *Streptococcus suis* Serotype 9. *PloS One*, 8(4), e61339.

Department of Environment, Food and Rural Affairs (2013) *Zoonoses report UK 2012*. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/236983/pb13987-zoonoses-report-2012.pdf [Accessed 12 August 2014].

Department of livestock development (2012) *Animal health in Thailand 2011* [online] p.pp. 86. Available from: http://pvlo-lbr.dld.go.th/eng/doc/animalhealthinthailand2011.pdf [Accessed 13 February 2016].

Department of Livestock Development (2014) *Livestock statistical Thailand 2014* [online]. [Accessed 14 December 2016].

Devriese, L.A. and Haesebrouck, F. (1992) *Streptococcus suis* infections in horses and cats. *Veterinary Record*. 130(17), 380–380.

Devriese, L.A., Haesebrouck, F., Herdt, P. de, Dom, P., Ducatelle, R., Desmidt, M., Messier, S. and Higgins, R. (1994) *Streptococcus suis* infections in birds. *Avian Pathology*. 23(4), 721-724.

DiCicco-Bloom, B. and Crabtree, B.F. (2006) The qualitative research interview. *Medical Education*. 40(4), 314-321.

Dickie, A.S., Bremner, D.A., Wong, P.Y., North, J.D. and Robertson, I.D. (1987) *Streptococcus suis* bacteraemia. *The New Zealand Medical Journal*. 100 (835), 677-678.

Division of livestock extension and development (n.d.) Training on sustainable backyard and small-scale pig raising. Available from: http://extension.dld.go.th/th1/ [Accessed 10 April 2016].

DLD (2011) *Summary on pigs and pig holders' data and statistic 2011* [online] p.pp. 14. Available from: http://ict.dld.go.th/th/images/stories/stat_web/yearly/2554/pig54/report_pig_54.pdf [Accessed 25 January 2016].

Donev, D., Zaletel-Kragelj, L., Bjegovic, V. and Burazeri, G. (2010) Measuring the burden of disease: disability adjusted life year (DALY). *Methods and Tools in Public Health*. 30(715), pp 30.

Donsakul, K., Dejthevaporn, C. and Witoonpanich, R. (2003a) *Streptococcus suis infection: clinical features and diagnostic pitfalls.* Available from: http://imsear.li.mahidol.ac.th/handle/123456789/31991 [Accessed 23 July 2015].

Doudoulakakis, A.G., Bouras, D., Drougka, E., Kazantzi, M., Michos, A., Charisiadou, A., Spiliopoulou, I., Lebessi, E. and Tsolia, M. (2016) Community-associated Staphylococcus aureus pneumonia among Greek children: epidemiology, molecular characteristics, treatment, and outcome. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology*. 35(7), 1177-85. doi: 10.1007/s10096-016-2651-7.

Dragojlović, J., Milosević, B., Sasić, N., Pelemis, M. and Sasić, M. (2005) *Streptococcus suis* infection--clinical manifestations. *Medicinski Pregled*. 58 (5-6), 236-239.

Du, H., Huang, W., Xie, H., Ye, C., Jing, H., Ren, Z. and Xu, J. (2013) The genetically modified suilysin, rSLYP353L, provides a candidate vaccine that suppresses proinflammatory response and reduces fatality following infection with *Streptococcus suis*. *Vaccine*. 31(38), 4209-4215.

Euser, A.M., Zoccali, C., Jager, K.J. and Dekker, F.W. (2009) Cohort Studies: Prospective versus Retrospective. *Nephron Clinical Practice*. 113(3), 214-217.

Falvey, J.L. and Visitpanich, T. (1980) A Survey of the Highland Pig Industry. *Thai Journal of Agricultural Science*. 13, 259-267.

FAO (n.d.) *Manual on meat inspection for developing countries*. Available from: http://www.fao.org/docrep/003/t0756e/t0756e02.htm [Accessed 21 April 2016].

FAO (2011) Swine industry profile of selected South East Asian Countries [online]. Bangkok: Food and Agriculture Organization of the United Nations, Regional Office fro Asia and the Pacific. [Accessed 12 February 2017].

Farid, M.N. and Frerichs, R.R. (2007) Software and Manual CSurvey version 2.0 [online]. Available from: http://www.ph.ucla.edu/epi/programs/csurvey2_manual.pdf [Accessed 28 June 2013].

Feng, P., Tan, M., Chen, Z., Xue, X., Tang, H., Liu, Z., Ma, J., Zhong, M. and Yuan, S. (2007) [Clinical features and outcome of infection of type 2 *Streptococcus suis* in human]. *Journal of Sichuan University. Medical Science Edition*. 38(5), 874-878.

Feng, W., Laster, S.M., Tompkins, M., Brown, T., Xu, J.-S., Altier, C., Gomez, W., Benfield, D. and McCaw, M.B. (2001) *In Utero* Infection by Porcine Reproductive and Respiratory Syndrome Virus Is Sufficient to Increase Susceptibility of Piglets to Challenge by *Streptococcus suis* Type II. *Journal of Virology*. 75(10), 4889-4895.

Ferrando, M.L. and Schultsz, C. (2016) A hypothetical model of host-pathogen interaction of *Streptococcus suis* in the gastro-intestinal tract. *Gut Microbes*. 7(2), 154-162.

Fittipaldi, N., Segura, M., Grenier, D. and Gottschalk, M. (2012) Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. *Future Microbiology*. 7(2), 259-279. doi:10.2217/fmb.11.149.

Fittipaldi, N., Xu, J., Lacouture, S., Tharavichitkul, P., Osaki, M., Sekizaki, T., Takamatsu, D. and Gottschalk, M. (2011) Lineage and virulence of *Streptococcus suis* serotype 2 isolates from North America. *Emerging Infectious Diseases*. 17(12), 2239–2244.

Foege W (1994) PReventive medicine and public health. *JAMA*. 271(21), 1704-1705.

Fongcom, A., Pruksakorn, S., Mongkol, R., Tharavichitkul, P. and Younim, N. (2001) *Streptococcus sui*s infection in northern Thailand. *Annual of Medicine*. 84(10), 1502-1508.

Fongcom, A., Pruksakorn, S., Netsirisawan, P., Pongprasert, R. and Onsibud, P. (2009) *Streptococcus suis* infection: a prospective study in northern Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*. 40(3), 511-517.

Fowler, H.N., Brown, P., Rovira, A., Shade, B., Klammer, K., Smith, K. and Scheftel, J. (2013) *Streptococcus suis* Meningitis in Swine Worker, Minnesota, USA. *Emerging Infectious Diseases*. 19(2), 330-331.

Foxman, B. and Riley, L. (2001) Molecular Epidemiology: Focus on Infection. *American Journal of Epidemiology*. 153(12), 1135-1141.

Fratamico P.M., Arun K. Bhunia, and J. L. Smith (eds.) (2005) *Foodborne Pathogens: Microbiology and Molecular Biology*. Wymondham, Norofolk, UK: Caister Academic Press. doi:10.3201/eid1212.061077

Friis, R.H. (2010) Epidemiology 101. Sudbury, Massachusette, Jones & Bartlett Learning. pp. 220.

Galina, L., Pijoan, C., Sitjar, M., Christianson, W.T., Rossow, K. and Collins, J.E. (1994) Interaction between *Streptococcus suis* serotype 2 and porcine reproductive and respiratory syndrome virus in specific pathogen-free piglets. *The Veterinary Record*. 134(3), 60-64.

Gao, X., Xiao, J., Qin, H., Cao, Z. and Wang, H. (2016) Impact of meteorological factors on the prevalence of porcine pasteurellosis in the southcentral of Mainland China. *Preventive Veterinary Medicine*. 125, 75-81.

Gibbons, C.L., Mangen, M.-J.J., Plass, D., Havelaar, A.H., Brooke, R.J., Kramarz, P., Peterson, K.L., Stuurman, A.L., Cassini, A., Fèvre, E.M. and Kretzschmar, M.E. (2014) Measuring underreporting and under-ascertainment in infectious disease datasets: a comparison of methods. *BMC Public Health*. 14, 147.

Gill, P., Stewart, K., Treasure, E. and Chadwick, B. (2008) Methods of data collection in qualitative research: interviews and focus groups. *British Dental Journal*. 204(6), 291-295.

Gómez-Zorrilla, S., Ardanuy, C., Lora-Tamayo, J., Cámara, J., García-Somoza, D., Peña, C. and Ariza, J. (2014) *Streptococcus suis* Infection and Malignancy in Man, Spain. *Emerging Infectious Diseases*. 20(6), 1067-1068.

Gottschalk, M. (2004) Diagnostic Notes: Porcine *Streptococcus suis* strains as potential sources of infections in humans: an underdiagnosed problem in North America? *Journal Swine Health Production.* 12(4), 197-199.

Gottschalk, M. (2010) *Streptococcus suis* Infection*:* Streptococcal Infections in Pigs*: Merck Veterinary Manual*. In: 10th edition [online]. Kenilworth, N.J.: Merck. Available from: http://www.merckvetmanual.com/mvm/generalized_conditions/streptococcal_infections_in_pigs/streptococcus_suis_infection.html [Accessed 11 February 2016].

Gottschalk, M. (2014) *Streptococcus suis* Infection*:* Streptococcal Infections in Pigs. Available from: http://www.merckvetmanual.com/mvm/generalized_conditions/streptococcal_infections_in_pigs/streptococcus_suis_infection.html [Accessed 9 May 2016].

Gottschalk, M., Higgins, R. and Beaudoin, M. (1990) Quebec. Distribution of *Streptococcus suis* capsular types 9 to 22 according to the site of isolation. *The Canadian Veterinary Journal*. 31(5), 393.

Gottschalk, M., Higgins, R., Jacques, M., Beaudoin, M. and Henrichsen, J. (1991) Characterization of six new capsular types (23 through 28) of *Streptococcus suis*. *Journal of Clinical Microbiology*. 29(11), 2590-2594.

Gottschalk, M., Higgins, R., Jacques, M., Mittal, K.R. and Henrichsen, J. (1989) Description of 14 new capsular types of *Streptococcus suis*. *Journal of Clinical Microbiology*. 27(12), 2633-2636.

Gottschalk, M., Kerdsin, A., Oishi, K., Hamada, S., Takeuchi, D. and Akeda, Y. (2014) A human case of *Streptococcus suis* infection caused by an unencapsulated strain. *JMM Case Reports* 1(2), doi: 10.1099/jmmcr.0.002329 Available from: http://jmmcr.microbiologyresearch.org/content/journal/jmmcr/10.1099/jmmcr.0.002329 [Accessed 8 December 2015].

Gottschalk, M., Lacouture, S., Bonifait, L., Roy, D., Fittipaldi, N. and Grenier, D. (2013) Characterization of *Streptococcus suis* isolates recovered between 2008

and 2011 from diseased pigs in Québec, Canada. *Veterinary Microbiology*. 162(24), 819-825.

Gottschalk, M., Lacouture, S. and Odierno, L. (1999) Immunomagnetic isolation of *Streptococcus suis* serotypes 2 and 1/2 from swine tonsils. *Journal of Clinical Microbiology*. 37(9), 2877-2881.

Gottschalk, M. and Segura, M. (2000) The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. *Veterinary microbiology*. 76(3), 259-272.

Gottschalk, M., Xu, J., Calzas, C. and Segura, M. (2010) *Streptococcus suis*: a new emerging or an old neglected zoonotic pathogen? *Future Microbiology*. 5(3), 371-391.

Goyette-Desjardins, G., Auger, J.-P., Xu, J., Segura, M. and Gottschalk, M. (2014) *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. *Emerging Microbes & Infections*. 3(6), e45. doi: 10.1038/emi.2014.45

Gray, L.D. and Fedorko, D.P. (1992) Laboratory diagnosis of bacterial meningitis. *Clinical Microbiology Reviews*. 5(2), 130-145.

Gregg, M.B. (2008) *Field Epidemiology*. 3rd edition. Oxford, New York. Oxford University Press. pp. 592.

Grosso-Becera, M.V., Servín-González, L. and Soberón-Chávez, G. (2015) RNA structures are involved in the thermoregulation of bacterial virulence-associated traits. *Trends in Microbiology*. 23(8), 509-518.

Haleis, A., Alfa, M., Gottschalk, M., Bernard, K., Ronald, A. and Manickam, K. (2009) Meningitis caused by *Streptococcus suis* serotype 14, North America. *Emerging Infectious Diseases*. 15(2), 350-352.

Hatrongjit, R., Kerdsin, A., Gottschalk, M., Takeuchi, D., Hamada, S., Oishi, K. and Akeda, Y. (2015) First human case report of sepsis due to infection with *Streptococcus suis* serotype 31 in Thailand. *BMC Infectious Diseases*. 15. 392. doi 10.1186/s12879-015-1136-0.

Health statistic plan subcommitee (2013) Health Statistic Plan 2014-2015 [online]. Available from: http://osthailand.nic.go.th/files/social_sector/SDP_health291057-new6.pdf [Accessed 22 January 2016].

Heidt, M.C., Mohamed, W., Hain, T., Vogt, P.R., Chakraborty, T. and Domann, E. (2005) Human Infective Endocarditis Caused by *Streptococcus suis* Serotype 2. *Journal of Clinical Microbiology*. 43(9), 4898-4901.

Hewison, K. (2014) Thailand: The Lessons of Protest. *Journal of Critical Perspectives on Asia*. 50(1), 1-15.

Higgins, R., Gottschalk, M., Boudreau, M., Lebrun, A. and Henrichsen, J. (1995) Description of Six New Capsular Types (29–34) of *Streptococcus suis*. *Journal of Veterinary Diagnostic Investigation*. 7(3), 405-406.

Higgins, R., Gottschalk, M., Fecteau, G., Sauvageau, R., De Guise, S. and Du Tremblay, D. (1990) Quebec. Isolation of *Streptococcus suis* from cattle. *The Canadian Veterinary Journal*. 31(7), 529.

Hill, J.E., Gottschalk, M., Brousseau, R., Harel, J., Hemmingsen, S.M. and Goh, S.H. (2005) Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that Streptococcus suis serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti*. *Veterinary Microbiology*. 107(1-2), 63-69.

Hmonpangtiam, K. and Chuknam, T. (2011) Situation of *Streptococcus suis* in Thailand, 2010. *Weekly Epidemiological Surveillance Report, Thailand*. 42(35), 549-551.

Ho, D.T.N., Le, T.P.T., Wolbers, M., Cao, Q.T., Nguyen, V.M.H., Tran, V.T.N., Le, T.P.T., Nguyen, H.P., Tran, T.H.C., Dinh, X.S., To, S.D., Hoang, T.T.H., Hoang, T., Campbell, J., et al. (2011) Risk Factors of *Streptococcus suis* Infection in Vietnam. A Case-Control Study. *PLoS One*, 6(3), e17604. doi: 10.1371/journal.pone.0017604.

Hoa, N.T., Chieu, T.T.B., Dong, S.D., Long, N.T., Hieu, T.Q., Luc, N.T. and Nhuong, P.T. (2013) *Streptococcus suis* and Porcine reproductive and respiratory syndrome, Vietnam. *Emerging Infectious Diseases*. 19(2), 331-332.

Hoa, N.T., Chieu, T.T.B., Nghia, H.D.T., Mai, N.T.H., Anh, P.H., Wolbers, M., Baker, S., Campbell, J.I., Chau, N.V.V., Hien, T.T., Farrar, J. and Schultsz, C. (2011) The antimicrobial resistance patterns and associated determinants in *Streptococcus suis* isolated from humans in southern Vietnam, 1997-2008. *BMC Infectious Diseases*. 11, 6. doi: 10.1186/1471-2334-11-6.

Holtz, C. (2012) *Global Health Care*. Burlington, Massachusetts, Jones & Bartlett Publishers. pp.643.

Honda, K. and Littman, D.R. (2012) The microbiome in infectious disease and inflammation. *Annual Review of Immunology*. 30, 759-795.

Hong Kong Special Administration Region Government (2005) *Press release: Streptococcus suis* to become notifiable disease. Available from: http://www.info.gov.hk/gia/general/200508/01/08010300.htm [Accessed 12 August 2014].

Horby, P., Wertheim, H., Ha, N.H., Trung, N.V., Trinh, D.T., Taylor, W., Ha, N.M., Lien, T.T.M., Farrar, J. and Van Kinh, N. (2010) Stimulating the development

of national *Streptococcus suis* guidelines in Viet Nam through a strategic research partnership. *Bulletin of the World Health Organization*. 88(6), 458-461.

Hoza, A.S., Mfinanga, S.G., Moser, I. and König, B. (2016) Molecular characterization of *Mycobacterium tuberculosis* isolates from Tanga, Tanzania: First insight of MIRU-VNTR and microarray-based spoligotyping in a high burden country. *Tuberculosis (Edinburgh, Scotland)*. 98,116-124.

Hsueh, K.J., Lee, J.W., Hou, S.M., Chen, H.S., Chang, T.C. and Chu, C.Y. (2014) Evaluation on a *Streptococcus suis* Vaccine Using Recombinant Sao-L Protein Manufactured by Bioreactors as the Antigen in Pigs. *Transboundary and Emerging Diseases*. 61(6), e35-e43.

Huh, H.J., Park, K.J., Jang, J.H., Lee, M., Lee, J.H., Ahn, Y.H., Kang, C.I., Ki, C.S. and Lee, N.Y. (2011) *Streptococcus suis* Meningitis with Bilateral Sensorineural Hearing Loss. *The Korean Journal of Laboratory Medicine*. 31 (3), 205-211.

Hui, A.C.F., Ng, K.C., Tong, P.Y., Mok, V., Chow, K.M., Wu, A. and Wong, L.K.S. (2005) Bacterial meningitis in Hong Kong: 10-years' experience. *Clinical Neurology and Neurosurgery*. 107(5), 366-370.

Huong, V.T.L., Ha, N., Huy, N.T., Horby, P., Nghia, H.D.T., Thiem, V.D., Zhu, X., Hoa, N.T., Hien, T.T., Zamora, J., Schultsz, C., Wertheim, H.F.L. and Hirayama, K. (2014) Epidemiology, Clinical Manifestations, and Outcomes of *Streptococcus suis* Infection in Humans. *Emerging Infectious Diseases* 20(7), 1105-1114.

IBP (2013) *Thailand Tax Guide Volume 1 Strategic and Practical Information*. Int'l Business Publications, Inc. pp. 283.

International Health Policy Program (2004) *Disabillity-Adjusted Life Year study in Congenital anomalies* [online] p.pp. 13. Available from: http://www.hiso.or.th/hiso/picture/reportHealth/pro2-chapter8(2).pdf [Accessed 9 May 2016].

International Health Policy Program (2011a) Burden of disease Thailand 2011 [online] p.pp. 60. Available from: http://social.nesdb.go.th/SocialStat/StatReport_Final.aspx?reportid=879&template=1R2C&yeartype=M&subcatid=21 [Accessed 22 January 2016].

International Health Policy Program (2011b) Regional differences in burden of disease in Thailand 2011 p.pp. 68.

International Health Policy Program (2015) Disability-Adjusted Life Year*:* DALY Report of Disability Adjusted Life Year and Injuries of Thai population 2013 [online] p.pp. 53. Available from:

http://ihppthaigov.net/DB/publication/attachresearch/372/chapter1.pdf [Accessed 8 May 2016].

Jansen, E. and Van Dorssen, C. (1951) Meningoencephalitis bij varkens door streptococcen. *Tijdschr Diergeneeskd*. 76,815-832.

Jaturpahu, U. (2009) Outbreak of *Streptococcus suis* serotype 2 in Chiang Mai, Lamphun Province, June-July 2009. *Surveillance Report Thailand*. 40(38), 633-640.

Jiang, X., Yang, Y., Zhu, L., Gu, Y., Shen, H., Shan, Y., Li, X., Wu, J. and Fang, W. (2016) Live Streptococcus suis type 5 strain XS045 provides cross-protection against infection by strains of types 2 and 9. *Vaccine*. 34(51), 6529-6538.

Jones, C.O. and Williams, H.A. (2004) The social burden of malaria: what are we measuring? *The American Journal of Tropical Medicine and Hygiene*. 71(2) 156-161.

Kamolnavin, S. (n.d.) Agricultural in Rattankosin era. Available from: http://archives.psd.ku.ac.th/kuout/p011.html [Accessed 8 December 2015].

Kantu, A., Woradecha, K. and Niyompeng, S. (2008) *Streptococcus suis* in Amphur Muang, Phetchabun Province [online] pp. 3. Available from: http://203.157.15.4/wesr/file/y51/F51281.pdf [Accessed 3 January 2016].

Kateera, F., Nsobya, S.L., Tukwasibwe, S., Mens, P.F., Hakizimana, E., Grobusch, M.P., Mutesa, L., Kumar, N. and van Vugt, M. (2016) Malaria case clinical profiles and *Plasmodium falciparum* parasite genetic diversity: a cross sectional survey at two sites of different malaria transmission intensities in Rwanda. *Malaria Journal*. 15(1), 237. doi:10.1186/s12936-016-1287-5.

Kay, R., Cheng, A.F. and Tse, C.Y. (1995) *Streptococcus suis* infection in Hong Kong. *QJM: Monthly Journal of the Association of Physicians*. 88(1), 39-47.

Kerdsin, A., Akeda, Y., Hatrongjit, R., Detchawna, U., Sekizaki, T., Hamada, S., Gottschalk, M. and Oishi, K. (2014) *Streptococcus suis* serotyping by a new multiplex PCR. *Journal of Medical Microbiology*. 63 (6), 824-830.

Kerdsin, A., Dejsirilert, S., Akeda, Y., Sekizaki, T., Hamada, S., Gottschalk, M. and Oishi, K. (2012) Fifteen S*treptococcus suis* serotypes identified by multiplex PCR. *Journal of Medical Microbiology*. 61(12), 1669-1672.

Kerdsin, A., Dejsirilert, S., Puangpatra, P., Sripakdee, S., Chumla, K., Boonkerd, N., Polwichai, P., Tanimura, S., Takeuchi, D., Nakayama, T., Nakamura, S., Akeda, Y., Gottschalk, M., Sawanpanyalert, P., et al. (2011a) Genotypic Profile of *Streptococcus suis* Serotype 2 and Clinical Features of Infection in Humans, Thailand. *Emerging Infectious Diseases*. 17(5), 835–842.

Kerdsin, A., Dejsirilert, S., Sawanpanyalert, P., Boonnark, A., Noithachang, W., Sriyakum, D., Simkum, S., Chokngam, S., Gottschalk, M., Akeda, Y. and Oishi, K. (2011b) Sepsis and spontaneous bacterial peritonitis in Thailand. *The Lancet*. 378(9794), 960. doi:10.3389/fmicb.2011.00235

Kerdsin, A., Hatrongjit, R., Gottschalk, M., Takeuchi, D., Hamada, S., Akeda, Y. and Oishi, K. (2015) Emergence of *Streptococcus suis* serotype 9 infection in humans. *Journal of Microbiology, Immunology and Infection* [online]. Available from: http://www.sciencedirect.com/science/article/pii/S1684118215008221 [Accessed 21 April 2016].

Kerdsin, A., Oishi, K., Sripakdee, S., Boonkerd, N., Polwichai, P., Nakamura, S., Uchida, R., Sawanpanyalert, P. and Dejsirilert, S. (2009) Clonal dissemination of human isolates of *Streptococcus suis* serotype 14 in Thailand. *Journal of Medical Microbiology*. 58(11), 1508-1513.

Khadthasima, N., Sutdan, D., Noimoh, T., Chalamat, M., Thannnawitjaya, P. and Areechokechai, D. (2007) Outbreak investigation of *S. suis* in Phusang district, Phayao Province, May 2007. 38(23), 393-398.

Khadthasrima, N., Hannwong, T., Thammawitjaya, P., Pingsusean, D., Akkanij, B., Jaikhar, A., Paungmali, P. and Yudee, P. (2007a) *Human Streptococcus suis outbreak in Phayao province, Thailand, 2007* [online] p.pp. 3. Available from: http://www.osirjournal.net/upload/files/2_Streptococcus_suis_new.pdf [Accessed 3 January 2016].

Khamlar, R., Wichit, A., Srichan, Y., Imsamran, P. and Sripat, N. (2013) Risk communication model of deafness prevention owing to *Streptococcus suis* infection in Phusang district, Phayao province. *Lanna Health Journal,* 6(1), 104–114.

Khikhuntod, J., Duangsonk, K., Deeudom, M. and Thananchai, H. (2016) Phenotypic variation of *Streptococcus suis* Serotype 2 isolated from Northern Thai patients characterized by survival in human blood and serum. *Bulletin of Chiang Mai Associated Medical Sciences*. 49(3), 377.

King, S.J., Leigh, J.A., Heath, P.J., Luque, I., Tarradas, C., Dowson, C.G. and Whatmore, A.M. (2002) Development of a multilocus sequence typing scheme for the pig pathogen *Streptococcus suis*: identification of virulent clones and potential capsular serotype exchange. *Journal of Clinical Microbiology*. 40(10), 3671-3680.

Kitpati, R. (n.d.) Development of Surveillance, control and prevention system under One Health Concept [online]. Available from: http://www.thaionehealth.org/uploads/file/one%20healthp9-15.pdf [Accessed 9 December 2015].

Klipper-Balz, R. and Schleifer, K. (1987) *Streptococcus suis* sp. nov. rev. *International Journal of Systtematic Bacteriology*. 37, 160-162.

Koch, E., Fuentes, G., Carvajal, R., Palma, R., Aguirre, V., Cruz, C., Henríquez, R. and Calvo, M. (2013) *Streptococcus suis* meningitis in pig farmers: report of first two cases in Chile. *Revista Chilena De Infectología: Órgano Oficial De La Sociedad Chilena De Infectología*. 30(5), 557-561.

Kongkaew W. (2011) *Characterisation of factors influencing trichinellosis in humans and pigs in Nan procince, northern Thailand*. Thesis (PhD), School of Biomedical Sciences, The University of Edinburgh.

Kopić, J., Paradzik, M.T. and Pandak, N. (2002) *Streptococcus suis* infection as a cause of severe illness: 2 cases from Croatia. *Scandinavian Journal of Infectious Diseases*. 34(9), 683-684.

Kotsuki, S., Tanaka, K. and Komori, D. (no date) Long-term Water Balance Analysis Using Different Precipitation Products In Upper Chao. *JICA (IMPAC-T)* [online]. Available from: http://www.kotsuki-shunji.com/document/paper/2013.08.aphw.kotsuki.pdf [Accessed 22 January 2016].

Kunnalok, T. (1996) *Food habits related to Opisthorchiasis of people in Mee Khan Lan Tham Village, Song Kwae sub-district, Chom Thong District, Chiang Mai Province*. Thesis (Master), Chiang Mai University.

Lakkitjaroen, N., Kaewmongkol, S., Karnchanabanthoeng, A., Satchasataporn, K., Abking, N. and Rerkamnuaychoke, W. (2009) *Prevalence of Streptococcus suis isolated from slaughter pigs in Northern Thailand*. Available from: http://vettech.ku.ac.th/iresearch/photo/5.pdf [Accessed 13 June 2013].

Lakkitjaroen, N., Karnchanabanthoeng, A., Kaewmongkol, S. and Metheenukul, P. (2008) Five most important zoonoses in Thailand. In: *16 years Faculty of veterinary technique, Kasetsart University* [online]. (no place) Kasetsart University, Faculty of veterinary technique. pp. 47–53. Available from: http://kukr.lib.ku.ac.th/db/BKN_VETTECH/search_detail/result/162671 [Accessed 13 April 2016].

Lalonde, M., Segura, M., Lacouture, S. and Gottschalk, M. (2000) Interactions between *Streptococcus suis* serotype 2 and different epithelial cell lines. *Microbiology.* 146(8) 1913-1921.

Lapointe, L., D'Allaire, S., Lebrun, A., Lacouture, S. and Gottschalk, M. (2002) Antibody response to an autogenous vaccine and serologic profile for *Streptococcus suis* capsular type 1/2. *Canadian Journal of Veterinary Research.* 66(1), 8-14.

Larsen, M.V., Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R.L., Jelsbak, L., Sicheritz-Pontén, T., Ussery, D.W., Aarestrup, F.M. and Lund, O.

(2012) Multilocus Sequence Typing of Total-Genome-Sequenced Bacteria. *Journal of Clinical Microbiology*. 50(4), 1355-1361.

Lederberg, J. and McCray, A. (2001) Ome sweet omics-agenealogical treasury of words. *Scientist*. 15(8).8-10.

Leelarasamee, A., Nilakul, C., Tien-Grim, S., Srifuengfung, S. and Susaengrat, W. (1997) Streptococcus suis toxic-shock syndrome and meningitis. *Journal of the Medical Association of Thailand, Chotmaihet Thangphaet*. 80(1), 63-68.

Lin, X., Huang, C., Shi, J., Wang, R., Sun, X., Liu, X., Zhao, L. and Jin, M. (2015) Investigation of Pathogenesis of H1N1 Influenza Virus and Swine *Streptococcus suis* Serotype 2 Co-Infection in Pigs by Microarray Analysis. *PLoS One*, 10(4), e0124086.

Littman, D.R. and Pamer, E.G. (2011) Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell Host and Microbe*. 10(4), 311-323.

Liu, Q., Cao, L. and Zhu, X.-Q. (2014) Major emerging and re-emerging zoonoses in China: a matter of global health and socioeconomic development for 1.3 billion. *International Journal of Infectious Diseases*. 25, 65-72.

Liu, Z., Zheng, H., Gottschalk, M., Bai, X., Lan, R., Ji, S., Liu, H. and Xu, J. (2013) Development of Multiplex PCR Assays for the Identification of the 33 Serotypes of *Streptococcus suis. PLoS One*. 8(8), e72070.

Lun, Z.-R., Wang, Q.-P., Chen, X.-G., Li, A.-X. and Zhu, X.-Q. (2007) *Streptococcus suis*: an emerging zoonotic pathogen. *The Lancet Infectious Diseases*. 7(3), 201–209.

Ma, E., Chung, P.H., So, T., Wong, L., Choi, K.M., Cheug, D.T., Kam, K.M., Chuang, S.K. and Tsang, T. (2008) *Streptococcus suis* infection in Hong Kong: an emerging infectious disease? *Epidemiology and Infection*. 136(12), 1691-1697.

Madsen, L.W., Svensmark, B., Elvestad, K., Aalbaek, B. and Jensen, H.E. (2002) *Streptococcus suis* serotype 2 infection in pigs: new diagnostic and pathogenetic aspects. *Journal of Comparative Pathology*. 126(1), 57-65.

Mai, N.T.H., Hoa, N.T., Nga, T.V.T., Linh, L.D., Chau, T.T.H., Sinh, D.X., Phu, N.H., Chuong, L.V., Diep, T.S., Campbell, J., Nghia, H.D.T., Minh, T.N., Chau, N.V.V., de Jong, M.D., et al. (2008) *Streptococcus suis* meningitis in adults in Vietnam. *Clinical Infectious Diseases,* 46(5), 659-667.

Maiden, M.C.J. (2006) Multilocus Sequence Typing of Bacteria. *Annual Review of Microbiology*. 60(1), 561-588.

Maiden, M.C.J., Bygraves, J.A., Feil, E., Morelli, G., Russell, J.E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D.A., Feavers, I.M., Achtman, M. and Spratt, B.G. (1998) Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America*. 95(6), 3140-3145.

Maneerat, K., Yongkiettrakul, S., Kramomtong, I., Tongtawe, P., Tapchaisri, P., Luangsuk, P., Chaicumpa, W., Gottschalk, M. and Srimanote, P. (2013) Virulence Genes and Genetic Diversity of *Streptococcus suis* Serotype 2 Isolates from Thailand. *Transboundary and Emerging Diseases*. 60, 69-79.

Mann, C.J. (2003) Observational research methods. Research design II: cohort, cross sectional, and case-control studies. *Emergency Medicine Journal*. 20(1), 54-60.

Markowska-Daniel, I., Urbaniak, K., Stepniewska, K. and Pejsak, Z. (2010) Antibiotic susceptibility of bacteria isolated from respiratory tract of pigs in Poland between 2004 and 2008. *Polish Journal of Veterinary Sciences*. 13(1), 29-36.

Marois, C., Bougeard, S., Gottschalk, M. and Kobisch, M. (2004) Multiplex PCR Assay for Detection of *Streptococcus suis* Species and Serotypes 2 and 1/2 in Tonsils of Live and Dead Pigs. *Journal of Clinical Microbiology*. 42(7), 3169-3175.

Martín, V., Vela, A.I., Gilbert, M., Cebolla, J., Goyache, J., Domínguez, L. and Fernández-Garayzábal, J.F. (2007) Characterization of *Aerococcus viridans* Isolates from Swine Clinical Specimens. *Journal of Clinical Microbiology*. 45(9), 3053-3057.

Mathewson, N.D., Jenq, R., Mathew, A.V., Koenigsknecht, M., Hanash, A., Toubai, T., Oravecz-Wilson, K., Wu, S.-R., Sun, Y., Rossi, C., Fujiwara, H., Byun, J., Shono, Y., Lindemans, C., et al. (2016) Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nature Immunology*. 17(5), 505-513.

McLendon, B.F., Bron, A., J. and Mitchell, C.J. (1978) *Streptococcus suis* type II (group R) as a cause of endophthalmitis. *British Journal of Ophthalmology*. 62, 729-731.

Meng, F., Wu, N.H., Nerlich, A., Herrler, G., Valentin-Weigand, P. and Seitz, M. (2015) Dynamic Virus-Bacterium Interactions in a Porcine Precision-Cut Lung Slice Coinfection Model: Swine Influenza Virus Paves the Way for *Streptococcus suis* Infection in a Two-Step Process. *Infection and Immunity*. 83(7), 2806-2815.

Messier, S., Lacouture, S. and Gottschalk, M. (2008) Distribution of *Streptococcus suis* capsular types from 2001 to 2007. *The Canadian Veterinary Journal*. 49(5), 461-462.

Miller, R.A. (1996) The aging immune system: primer and prospectus. *Science,* 273(5271), 7074.

Minichiello, V., Aroni, R., Timewell, E. and Alexander, L. (1992) In-depth Interviewing*: Researching People*. South Melbourne: Routledge. pp. 357.

Ministry of Social development and human security (2013) *Thai ageing population: past and future* [online]. Available from: http://www.m-society.go.th/ewtadmin/ewt/mso_web/article_attach/13225/17347.pdf [Accessed 21 January 2016].

Ministry of Social development and human security (2015) *GDP 2014 and prediction for 2015* [online]. Available from: http://www.nesdb.go.th/Portals/0/eco_datas/economic/eco_state/4_57/PPTQ4-2014.pdf [Accessed 22 January 2016].

Moonchana, P. (2006) A comparative analysis of the Thai household expenditure pattern before and after the economic crisis in 1997. Master of Economic. [online]. Bangkok: University of Thai chamber of commerce. Available from: http://eprints.utcc.ac.th/3797/1/202001.pdf [Accessed 22 January 2016].

Morabia, A. (2005) *A History of Epidemiologic Methods and Concepts*. Springer Science & Business Media. pp. 405.

Morgan, L. (2014) Knowledge and Behavior Change in Children Within the University of Missouri Extension Family Nutrition Program. *Journal of Nutrition Education and Behavior*. 46(4), S149–S150.

Morrow-Teshch, J.L., McGlone, J.J. and Salak-Johnson, J.L. (1994) Heat and social stress effects on pig immune measures. *Journal of Animal Science.* 72(10), 2599-2609

Muckle, A., Giles, J. Lund, L., Stewart, T. and Gottschank, M. (2010) Isolation of *Streptococcus suis* from the unine of clinically ill dog. *The Canadian Veterinary Journal.* 51(7), 773-774. Murray C. and World Health Organization (eds.) (2002) Summary measures of population health: concepts, ethics, measurement, and applications. Geneva: World Health Organization. pp. 29.

Murray, C.J. (1994) Quantifying the burden of disease: the technical basis for disability-adjusted life years. *Bulletin of the World Health Organization*. 72 (3), 429-445.

Murray, C.J., Lopez, A.D. and Jamison, D.T. (1994) The global burden of disease in 1990: summary results, sensitivity analysis and future directions. *Bulletin of the World Health Organization*. 72(3), 495–509.

Nakayama, T., Takeuchi, D., Matsumura, T., Akeda, Y., Fujinaga, Y. and Oishi, K. (2013) Alcohol consumption promotes the intestinal translocation of *Streptococcus suis* infections. *Microbial Pathogenesis*. 65, 14-20.

National Statistic Office (n.d.) Life expectancy at birth. Available from: http://social.nesdb.go.th/SocialStat/StatReport_Final.aspx?reportid=88&template=2R1C&yeartype=M&subcatid=4 [Accessed 21 January 2016].

National Statistic Office (2013) *Priliminary Report 2013 Agricultural Census*. Thailand. Available from: http://service.nso.go.th/nso/nsopublish/census/agriculture.html

National Statistic Office (2014a) Average years in education of people in each age group and their location. Available from: http://social.nesdb.go.th/SocialStat/StatReport_FullScreen.aspx?reportid=879&template=1R2C&yeartype=M&subcatid=21 [Accessed 3 February 2016].

National Statistic Office (2014b) *Statistical yearbook Thailand 2011* [online] p.pp. 572. Available from: http://service.nso.go.th/nso/nsopublish/pubs/syb_54/SYB_54_T.pdf [Accessed 22 January 2016].

National Statistic Office (2014c) *Summary: Labor survey 2014* [online] p.pp. 86. Available from: http://service.nso.go.th/nso/nsopublish/themes/files/lfs57/SumResult57.pdf [Accessed 22 January 2016].

National Statistical Office (2015) *Smoking and alcohol drinking behavior survey of Thai population 2014* [online] p.pp. 364. Available from: https://www.m-society.go.th/article_attach/13207/17336.pdf [Accessed 10 May 2016].

National health security office (2015) Universal coverage scheme budget management manual [online]. Available from: http://www.nhso.go.th/files/userfiles/file/Download/2016.pdf [Accessed 7 March 2016].

National Statistic Office (2016) *Life expectancy at birth*. Available from: http://social.nesdb.go.th/SocialStat/StatReport_Final.aspx?reportid=88&template=2R1C&yeartype=M&subcatid=4 [Accessed 21 January 2016].

Navacharoen, N., Chantharochavong, V., Hanprasertpong, C., Kangsanarak, J. and Lekagul, S. (2009) Hearing and vestibular loss in *Streptococcus sui*s infection

from swine and traditional raw pork exposure in northern Thailand. *The Journal of Laryngology and Otology*. 123(8), 857-862.

Neumann, E.J., Ramirez, A. and Schwartz, K.J. (2009) Swine disease manual. Perry, Iowa: American Association of Swine Veterinarians. pp. 333.

Neungmek, A. and Pathanasophon, P. (2011) Prevalence and risk factors of Streptoccus suis slaughtered pigs in Phayao Province, Thialand. *Epidemiolgical surveillance report*. 18 (July), pp 4-7.

Nga, T.V.T., Nghia, H.D.T., Tu, L.T.P., Diep, T.S., Mai, N.T.H., Chau, T.T.H., Sinh, D.X., Phu, N.H., Nga, T.T.T., Chau, N.V.V., Campbell, J., Hoa, N.T., Chinh, N.T., Hien, T.T., et al. (2011) Real-time PCR for detection of *Streptococcus suis* serotype 2 in cerebrospinal fluid of human patients with meningitis. *Diagnostic Microbiology and Infectious Disease*. 70(4-19), 461-467.

Nghia, H.D.T., Hoa, N.T., Linh, L.D., Campbell, J., Diep, T.S., Chau, N.V.V., Mai, N.T.H., Hien, T.T., Spratt, B., Farrar, J. and Schultsz, C. (2008) Human Case of *Streptococcus suis* Serotype 16 Infection. *Emerging Infectious Diseases*. 14(1), 155-157.

Nguyen, T.H.M., Tran, T.H.C., Thwaites, G., Ly, V.C., Dinh, X.S., Ho Dang, T.N., Dang, Q.T., Nguyen, D.P., Nguyen, H.P., To, S.D., Nguyen, van V.C., Nguyen, M.D., Campbell, J., Schultsz, C., et al. (2007) Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. *The New England Journal of Medicine*. 357(24), 2431-2440.

Nomoto, R., Maruyama, F., Ishida, S., Tohya, M., Sekizaki, T. and Osawa, R. (2015) Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22 and 26: *Streptococcus parasuis* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*. 65(2), 438-443.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*. 28(12), e63.

Oh, Y.J. and Song, S.H. (2012) A Case of *Streptococcus suis* Infection Causing Pneumonia with Empyema in Korea. *Tuberculosis and Respiratory Diseases*. 73(3), 178-181.

Okura, M., Lachance, C., Osaki, M., Sekizaki, T., Maruyama, F., Nozawa, T., Nakagawa, I., Hamada, S., Rossignol, C., Gottschalk, M. and Takamatsu, D. (2014) Development of a Two-Step Multiplex PCR Assay for Typing of Capsular Polysaccharide Synthesis Gene Clusters of *Streptococcus suis*. *Journal of Clinical Microbiology*. 52(5), 1714-1719.

Okura, M., Takamatsu, D., Maruyama, F., Nozawa, T., Nakagawa, I., Osaki, M., Sekizaki, T., Gottschalk, M., Kumagai, Y. and Hamada, S. (2013) Genetic Analysis of Capsular Polysaccharide Synthesis Gene Clusters from All

Serotypes of *Streptococcus suis*: Potential Mechanisms for the Generation of Capsular Variation. *Applied and environmental microbiology*. 79(8), 2796-806.

Okwumabua, O., O'Connor, M. and Shull, E. (2003) A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase. *FEMS microbiology letters*. 218(1), 79-84.

Okwumabua, O., Peterson, H., Hsu, H.-M., Bochsler, P. and Behr, M. (2017) Isolation and partial characterization of *Streptococcus suis* from clinical cases in cattle. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*. 29(2): 160-168.

Onishi, H., Sugawara, M., Okura, M., Osaki, M. and Takamatsu, D. (2012) Prevalence of *Streptococcus suis* genotypes in isolates from porcine endocarditis in East Japan. *The Journal of Veterinary Medical Science / The Japanese Society of Veterinary Science*. 74(12), 1681-1684.

Padungtod, P., Tharavichitkul, P., Junya, S., Chaisowong, W., Kadohira, M., Makino, S. and Sthitmatee, N. (2010) Incidence and presence of virulence factors of *Streptococcus suis* infection in slaughtered pigs from Chiang Mai, Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*. 41(6), 14541461.

Palmieri, C., Varaldo, P.E. and Facinelli, B. (2011) *Streptococcus suis*, an Emerging Drug-Resistant Animal and Human Pathogen. *Frontiers in Microbiology*. 2, 235. doi: 10.3389/fmicb.2011.00235.

Pan, Z., Ma, Y., Ma, J., Dong, W. and Yao, H. (2016) Acute meningitis of piglets and mice caused by co-infected with *Streptococcus suis* and *Aerococcus viridans*. *Microbial Pathogenesis*. pii: S0882-4010(16)30347-3. doi: 10.1016/j.micpath.2016.10.024. [Epub ahead of print]

Pana-ananpaiboon, P. (2011) *Marketing mix factors affecting pork meat retailers towards purchasing pork in Muang Chiang Mai district*. Thesis (Master of Business Administration). Chiang Mai: Chiang Mai University.

Papatsiros VG, V.D. (2011) *Streptococcus suis:* an important zoonotic pathogen for human - prevention aspects. *Veterinary World*. 4, 216-221.

Pathanasophon, P., Narongsak, W., Worarach, A., Yuwapanichsampan, S. and Sagarasaeranee, P. (2009) Prevalence of *Streptococcus suis* in pigs and pig farmers in 11 provinces in the East and the West of Thailand. *Journal of Thai Veterinary Association*. 60(1-3), 49-62.

Pathanasophon, P., Worarach, A., Narongsak, W., Yuwapanichsampan, S., Nuangmek, A., Sakdasirisathaporn, A., Chuxnum, T. (2013) Prevalence of *Streptococcus suis* in Tonsils of Slaughtered Pigs in Lampang and Phayao

Provinces, Thailand, 2009-2010. *Journal of Tropical Medicine & Parasitology*. 36(1), 8-14.

Patz, J.A., Confalonieri, U.E.C., Amerasinghe, F.P., Chua, K.B., Daszak, P., Hyatt, A.D., Molyneux, D., Thomson, M., Yameogo, L., Lazaro, M.M. and others (2005) Human health: ecosystem regulation of infectious diseases. *Ecosystems and Human Well-Being: Current State and Trends: Findings of the Condition and Trends Working Group of the Millennium Ecosystem Assessment*. pp. 391-415.

Patz, J.A., Githeko, A.K., McCarty, J.P., Hussein, S., Confalonieri, U., De Wet, N. and others (2003) Climate change and infectious diseases. *Climate Change and Human Health: Risks and Responses*. pp. 103-132.

Perch, B., Kristjansen, P. and Skadhauge, K. (1968) Group R Streptococci pathogenic for man: two cases of meningitis and one fatal case of sepsis. *Acta Path Microbiol Scand*. 74(1), 69-76.

Perch, B., Pedersen, K.B. and Henrichsen, J. (1983) Serology of capsulated streptococci pathogenic for pigs: six new serotypes of *Streptococcus suis*. *Journal of Clinical Microbiology*. 17(6), 993-996.

Perera, M., Whitehead, M., Molyneux, D., Weerasooriya, M. and Gunatilleke, G. (2007) Neglected Patients with a Neglected Disease? A Qualitative Study of Lymphatic Filariasis Mitchell Weiss (ed.). *PLoS Neglected Tropical Diseases*. 1(2), e128.

Pfeiffer, D. (2010) *Veterinary epidemiology: an introduction*. Chichester, U.K.; Ames, Iowa: Wiley-Blackwell. pp.62.

Phuapradit, P., Boongrid, P., Boonyakarnkul, S., Niramarnsakul, S., Ponglikitmonkol, S. and Vorachit, M. (1987) Meningitis caused by *Streptococcus suis*. *Intern Med*. 3: 120–122.

Prasatkul, P. and Wapattanawong, P. (2012) Demographic structure and change. In: *Thai Health 2012* Thai Health [online]. Bangkok: Institute for population and social research, Mahidol University. pp. 10–11. Available from: http://www.hiso.or.th/hiso/picture/reportHealth/ThaiHealth2012/thai2012_2.pdf [Accessed 21 January 2016].

Pratumsawad, T. (no date) *Thai politic analysis* [online]. Available from: http://rajapark.ac.th/2014/wp-content/uploads/2014/08/03-08-2557-16-05.pdf [Accessed 22 January 2016].

Princivalli, M.S., Palmieri, C., Magi, G., Vignaroli, C., Manzin, A., Camporese, A., Barocci, S., Magistrali, C. and Facinelli, B. (2009) Genetic diversity of *Streptococcus suis* clinical isolates from pigs and humans in Italy (2003-2007).

Euro surveillance: bulletin 14 (33). [Online]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/19712640. [Accessed 5 August 2016].

Prompichai, R. (1999) Northern Thai Culture Encyclopedia. In: *Northern Thai culture encyclopedia*. Bangkok: Thai Culture Encyclopedia Foundation, Thai Commercial Bank. pp. 5937–5944.

Promthong, S. (no date) *Swine Farm Management* [online]. Available from: http://e-book.ram.edu/e-book/a/AT328/AT328-1.pdf [Accessed 8 December 2015].

Quinn, P.J., Markey, B.K., Leonard, F.C., FitzPatrick, E.S. and Fanning, S. (2015) *Concise Review of Veterinary Microbiology*. John Wiley & Sons. pp.153.

Quinn, P.J., Markey, B.K., Leonard, F.C., FitzPatrick, E.S., Fanning, S. and Hartigan, P. (2011) *Veterinary Microbiology and Microbial Disease*. Iowa State University Press, Ames, Iowa, pp 536.

Ranjbar, R., Karami, A., Farshad, S., Giammanco, G.M. and Mammina, C. (2014) Typing methods used in the molecular epidemiology of microbial pathogens: a how to guide. *New Microbiologica*. 1(15), 1–15.

Rattanamaneekorn, S. and Oopakornrat, S. (2014) *Streptococcus suis* septicemia and meningitis: case report.Disease prevention and control 7 Journal, 12(4), pp. 19–29.

Reams, R.Y., Harrington, D.D., Glickman, L.T., Thacker, H.L. and Bowersock, T.L. (1996) Multiple Serotypes and Strains of *Streptococcus suis* Infected Swine Herds. *Journal of Veterinary Diagnostic Investigation*. 8(1), 119-121.

Reviews, C.T. (2015) *Essentials of Epidemiology in Public Health: Medicine, Healthcare*. Cram101 Textbook Reviews. pp.516.

Rhee, S.H., Pothoulakis, C. and Mayer, E.A. (2009) Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature Reviews. Gastroenterology & Hepatology*. 6(5), 306-314.

Robertson, I.D. and Blackmore, D.K. (1989) Occupational Exposure to Streptococcus suis Type 2. *Epidemiology and Infection*. 103(1), 157-164.

da Rocha E.B., M., Freire, F. da C.O., Erlan Feitosa Maia, F., Izabel Florindo Guedes, M. and Rondina, D. (2014) Mycotoxins and their effects on human and animal health. *Food Control*. 36(1), 159-165.

Rosendal, S., Breton, J., Henrichsen, J., Hilt, L. and Mitchell, W.R. (1986) Isolation of *Streptococcus suis* using a selective medium. *Canadian Journal of Veterinary Research*. 50(4), 537-539.

Round, J.L. and Mazmanian, S.K. (2009) The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews. Immunology*. 9(5), 313-323.

Rusmeechan, S. and Sribusara, P. (2008) *Streptococcus suis* meningitis: the newest serious infectious disease. *Medical Journal of the Medical Association of Thailand*. 91(5), 654-658.

Sakunphanit, T. (n.d.) *Thailand: Universal health care coverage through pluralistic approaches* [online]. Available from: http://www.ilo.org/wcmsp5/groups/public/---ed_protect/---soc_sec/documents/publication/wcms_secsoc_6612.pdf [Accessed 22 January 2016].

Salit-apiruk, N. (2012) *Standard and Guideline for Surveillance Rapid and Response Team*. Nonthaburi: Bureau of Epidemiology, Department of Disease Control. pp.180.

Salomon, J.A., Vos, T., Hogan, D.R., Gagnon, M., Naghavi, M., Mokdad, A., Begum, N., Shah, R., Karyana, M., Kosen, S., Farje, M.R., Moncada, G., Dutta, A., Sazawal, S., et al. (2012) Common values in assessing health outcomes from disease and injury: disability weights measurement study for the Global Burden of Disease Study 2010. *Lancet*, 380(9859), 2129-2143.

Samerchea, S. and Neungmek, A. (2008) *Epidemiology of Streptococcus suis infection in Phayao*. Available from: http://www.osirjournal.net/upload/files/11_Streptococcus_suis_thai.pdf [Accessed 10 September 2015].

Schultsz, C., Jansen, E., Keijzers, W., Rothkamp, A., Duim, B., Wagenaar, J.A. and van der Ende, A. (2012) Differences in the population structure of invasive *Streptococcus suis* strains isolated from pigs and from humans in The Netherlands. *PLoS One*. 7 (5), e33854.

Segura, M. (2009) *Streptococcus suis*: An Emerging Human Threat. *Journal of Infectious Diseases.* 199(1), 4-6.

Shneerson, J.M., Chattopadhyay, B., Murphy, M.F. and Fawcett, I.W. (1980) Permanent perceptive deafness due to *Streptococcus suis* type II infection. *The Journal of Laryngology and Otology*. 94(4), 425-427.

Silva, I. dos S. and International Agency for Research on Cancer (1999) Cancer epidemiology: principles and methods. Lyon, France: *International Agency for Research on Cancer*. pp. 441.

Silva, L.M.G., Baums, C.G., Rehm, T., Wisselink, H.J., Goethe, R. and Valentin-Weigand, P. (2006) Virulence-associated gene profiling of *Streptococcus suis* isolates by PCR. *Veterinary Microbiology*. 115(1-3), pp. 117-127.

Singhapreecha, C. (2014) Economy and Agriculture in Thailand. Available from: http://ap.fftc.agnet.org/ap_db.php?id=246 [Accessed 8 December 2015].

Sittiroj, C. (2011) Underline Factors Related to *Streptococcus suis* Infection in Lamphun Province. Lanna Public Health Journal. 6(3), 337-350.

Slattery, M.L. (2002) The science and art of molecular epidemiology. *Journal of Epidemiology and Community Healt*h. 56(10), 728-729.

Smith, H.E., van Bruijnsvoort, L., Buijs, H., Wisselink, H.J. and Smits, M.A. (1999a) Rapid PCR test for *Streptococcus suis* serotype 7. *FEMS Microbiology* Letters. 178(2), 265-270.

Smith, H.E., Veenbergen, V., van der Velde, J., Damman, M., Wisselink, H.J. and Smits, M.A. (1999b) The cps Genes of *Streptococcus suis* Serotypes 1, 2, and 9: Development of Rapid Serotype-Specific PCR Assays. J*ournal of Clinical Microbiology*. 37(10), 3146-3152.

Smith, T.C., Capuano, A.W., Boese, B., Myers, K.P. and Gray, G.C. (2008) Occupational Exposure to *Streptococcus suis* among US Swine Workers. *Emerging Infectious Diseases*. 14(12), 1925-1927.

Smith, T.C., Gebreyes, W.A., Abley, M.J., Harper, A.L., Forshey, B.M., Male, M.J., Martin, H.W., Molla, B.Z., Sreevatsan, S., Thakur, S., Thiruvengadam, M. and Davies, P.R. (2013) Methicillin-Resistant *Staphylococcus aureus* in Pigs and Farm Workers on Conventional and Antibiotic-Free Swine Farms in the USA. *PLoS One* 8(5), e63704. doi: 10.1371/journal.pone.0063704.

Snow, M. (2011) The contribution of molecular epidemiology to the understanding and control of viral diseases of salmonid aquaculture. *Veterinary Research*. 42(1): 56.

Solomon, J., Haagsma, J., Davis, A., de Noordhout, C.M., Polinder, S., Havelaar, A.H. and Cassini, A. (2015) Disability weights for the Global Burden of Disease 2013 study. *The Lancet Global Health*. 3(11), e712–e723.

Song, J.W. and Chung, K.C. (2010) Observational Studies: Cohort and Case-Control Studies. *Plastic and Reconstructive Surgery*. 126(6), 2234–2242.

Soonthornthada, A. (2015) Migrant workers in greater Mekong Sub-region. In: *Population and social diversity in Thailand 2015* [online]. Nakornprathom:

Institute for population and social research, Mahidol University. pp. 149–163. Available from: http://www.ms.ipsr.mahidol.ac.th/ConferenceXI/Download /Book/447-IPSR-Conference-A10-fulltext.pdf [Accessed 22 January 2016].

Spellman, F.R. and Stoudt, M.L. (2013) *The Handbook of Environmental Health*. Scarecrow Press. pp 441.

Staats, J.J., Feder, I., Okwumabua, O. and Chengappa, M.M. (1997) *Streptococcus suis*: past and present. *Veterinary Research Communications*. 21(6), 381-407.

Straw B.E. (ed.) (2006) *Diseases of swine*. 9th ed. Ames, Iowa: Blackwell Pub.pp 1153.

Swildens, B., Stockhofe-Zurwieden, N., van der Meulen, J., Wisselink, H.J., Nielen, M. and Niewold, T.A. (2004) Intestinal translocation of *Streptococcus suis* type 2 EF+ in pigs. *Veterinary Microbiology*. 103(1-2), 29–33.

Szmolka, A. and Nagy, B. (2013) Multidrug resistant commensal Escherichia coli in animals and its impact for public health. *Frontiers in Microbiology*. 4, 258.

Takamatsu, D., Wongsawan, K., Osaki, M., Nishino, H., Ishiji, T., Tharavichitkul, P., Khantawa, B., Fongcom, A., Takai, S. and Sekizaki, T. (2008) *Streptococcus suis* in Humans, Thailand. *Emerging Infectious Diseases*. 14(1), 181-183.

Takeuchi, D., Kerdsin, A., Pienpringam, A., Loetthong, P., Samerchea, S., Luangsuk, P., Khamisara, K., Wongwan, N., Areeratana, P., Chiranairadul, P., Lertchayanti, S., Petcharat, S., Yowang, A., Chaiwongsaen, P., et al. (2012) Population-Based Study of *Streptococcus suis* Infection in Humans in Phayao Province in Northern Thailand. *PLoS One* 7(2), e31265.

Tangcharoensathien, V., Limwattananon, S., Patcharanarumol, W., Thammatacharee, J., Jongudomsuk, P. and Sirilak, S. (2014) Achieving universal health coverage goals in Thailand: the vital role of strategic purchasing. *Health Policy and Planning*. 30(9): 1152-1161.

Tangcharoensathien, V., Prakongsai, P., Limwattananon, S., Patcharanarumol, W. and Jongudomsuk, P. (2009) *From Targeting to Universality: Lessons from the Health System in Thailand* [online]. Available from: http://papers.ssrn.com/abstract=1487640 [Accessed 16 January 2016].

Tarradas, C., Arenas, A., Maldonado, A., Luque, I., Miranda, A. and Perea, A. (1994) Identification of *Streptococcus suis* isolated from swine: proposal for biochemical parameters. *Journal of Clinical Microbiology*. 32(2), 578-580.

Tarradas, C., Perea, A., Vela, A.I., Goyache, J., Dominguez, L., Fernández-Garaizabal, J.F., Borge, C., Huerta, B. and Luque, I. (2004) Distribution of serotypes of

Streptococcus suis isolated from diseased pigs in Spain. *The Veterinary Record*. 154(21), 665-666.

Taylor, L.H., Latham, S.M. and Woolhouse, M.E. (2001) Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 356(1411), 983-989.

Teekakirikul, P. and Wiwanitkit, V. (2003) *Streptococcus suis* infection: overview of case reports in Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*. 34(2),178-183.

Teutsch, S.M. and Churchill, R.E. (2000) *Principles and Practice of Public Health Surveillance*. Oxford University Press. pp 443.

Thacker, S.B. and Berkelman, R.L. (1988) Public Health Surveillance in the United States. *Epidemiologic Reviews*. 10(1), 164-190.

Thai broiler processing exporters association (n.d.) *Thai broiler processing exporters association*. Available from: http://www.thaifranchisecenter.com/links/show.php?id=1240 [Accessed 22 January 2016].

Thanawongnuwech, R., Brown, G.B., Halbur, P.G., Roth, J.A., Royer, R.L. and Thacker, B.J. (2000) Pathogenesis of porcine reproductive and respiratory syndrome virus-induced increase in susceptibility to *Streptococcus suis* infection. *Veterinary Pathology*. 37(2), 143-152.

Thayawiwat, C., Wichaikham, O. and Painpringam, A. (2013) Streptococcus suis Infection in the Patients of Chiang Kham Hospital, Chiang Kham District, Phayao Province 2009-2011: Screening Test for *Streptococcus suis* Infection. *Journal of Preventive Medicine Association of Thailand.* 2(2), 119-134.

The National Economic and Social Development Plan (no date) *Average year of study, by age, and living area*. Available from: http://social.nesdb.go.th/SocialStat/StatReport_Final.aspx?reportid=879&template=1R2C&yeartype=M&subcatid=21 [Accessed 22 January 2016].

The National Economic and Social Development Plan (2012) *Cluster mapping to elevate the competition ability of producers.* pp. 278.

The office of SMEs Promotion (2014) *Situation of Small and Medium size SMEs 2014* [online]. Available from:http://www.sme.go.th/SiteCollectionDocuments/White%20Paper/2556/report01.pdf [Accessed 22 January 2016].

The Thailand food committee (2012) *Strategic framework for food management in Thailand*. S.l.: s.n.].

The World Bank (1993) *World Development Report 1993*World Development Report. [online]. The World Bank. [Accessed 21 January 2016].

Thomas J.C. and David J. Weber (eds.) (2001) *Epidemiologic methods for the study of infectious diseases*. Oxford; New York: Oxford University Press. pp.32.

Thrusfield, M.V. (2007) *Veterinary epidemiology*. 3rd editon. Ames, Iowa: Blackwell Science. pp 624.

Tien, L.H.T., Nishibori, T., Nishitani, Y., Nomoto, R. and Osawa, R. (2013) Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22, 26, and 33 based on DNA-DNA homology and sodA and recN phylogenies. *Veterinary Microbiology*. 162(2-4), 842-849.

Tophothai, T., Thaijinda, C., Kiddee, J., Areechokchai, D., Patcharanarumol, W. and Thammarangsi, T. (2013) Situation analysis of health-threatening factor, Disease control and prevention system according to the statute on National health system B.E. 2552 (2009) [online] p.pp. 59. Available from: http://ihppthaigov.net/DB/publication/attachresearch/327/chapter1.pdf [Accessed 21 January 2016].

Tramontana, A.R., Graham, M., Sinickas, V. and Bak, N. (2008) An Australian case of *Streptococcus suis* toxic shock syndrome associated with occupational exposure to animal carcasses. *Medical Journal of Australia*., 188 (9), 538-539.

UNESCO (2015) *Adult literacy rate, population 15+ year*. Available from: http://data.uis.unesco.org/Index.aspx?DataSetCode=EDULIT_DS&popupcustomise=true&lang=en# [Accessed 21 January 2016].

Urwin, R. and Maiden, M.C.J. (2003) Multi-locus sequence typing: a tool for global epidemiology. *Trends in Microbiology*. 11(10), 479-487.

Van Samkar, A., Brouwer, M.C., Schultsz, C., Van der Ende, A. and Van de Beek, D. (2015) Streptococcus suis meningitis in the Netherlands. *Journal of Infection* [online]. Available from: http://www.sciencedirect.com/science/article/pii/S0163445315002248 [Accessed 2 August 2015].

Vecht, U., Arends, J.P., van der Molen, E.J. and van Leengoed, L.A. (1989) Differences in virulence between two strains of *Streptococcus suis* type II after experimentally induced infection of newborn germ-free pigs. *American Journal of Veterinary Research*. 50(7), 1037-1043.

Vecht, U., Wisselink, H.J., van Dijk, J.E. and Smith, H.E. (1992) Virulence of *Streptococcus suis* type 2 strains in newborn germfree pigs depends on phenotype. *Infection and Immunity*. 60(2), 550-556.

Vilaichone, R.-K. (2002) *Streptococcus suis* infection in Thailand. *Annual of medicine*. (special 1), 109-111.

Voigt, K. and King, N.B. (2014) Disability weights in the global burden of disease 2010 study: two steps forward, one step back? *Bulletin of the World Health Organization*. 92(3), 226-228.

Vos, T., Flaxman, A.D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M. and Shibuya, K. (2012) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010, *Lancet*. 380, 2163-2196.

Voutsadakis, I.A. (2006) Streptococcus suis Endocarditis and Colon Carcinoma: A Case Report. *Clinical Colorectal Cancer*. 6(3), 226-228.

Wang, Y., Gagnon, C.A., Savard, C., Music, N., Srednik, M., Segura, M., Lachance, C., Bellehumeur, C. and Gottschalk, M. (2013) Capsular sialic acid of *Streptococcus suis* serotype 2 binds to swine influenza virus and enhances bacterial interactions with virus-infected tracheal epithelial cells. *Infection and Immunity*. 81(12), 4498-4508.

Wangkaew, S., Chaiwarith, R., Tharavichitkul, P. and Supparatpinyo, K. (2006) *Streptococcus suis* infection: a series of 41 cases from Chiang Mai University Hospital. *The Journal of Infection*. 52(6), 455-460.

Wei, Z., Li, R., Zhang, A., He, H., Hua, Y., Xia, J., Cai, X., Chen, H. and Jin, M. (2009) Characterization of *Streptococcus suis* isolates from the diseased pigs in China between 2003 and 2007. *Veterinary Microbiology*. 137(1-2), 196-201.

Wertheim, H.F.L., Nghia, H.D.T., Taylor, W. and Schultsz, C. (2009a) *Streptococcus suis*: an emerging human pathogen. *Clinical Infectious Diseases.* 48(5), 617-625.

Wertheim, H.F.L., Nguyen, H.N., Taylor, W., Lien, T.T.M., Ngo, H.T., Nguyen, T.Q., Nguyen, B.N.T., Nguyen, H.H., Nguyen, H.M., Nguyen, C.T., Dao, T.T., Nguyen, T.V., Fox, A., Farrar, J., et al. (2009b) *Streptococcus suis*, an important cause of adult bacterial meningitis in northern Vietnam. *PloS One*. 4(6), e5973.

WHO (1996) *Investing in Health Research and Developement* [online]. Available from: http://apps.who.int/iris/bitstream/10665/63139/1/TDR_GEN_96.2.pdf [Accessed 21 January 2016].

WHO (2001) National burden of disease studies: a practical guide. *World Health* [online]. Available from: http://www.thaibod.net/documents/NBD%20Manual%20V2.0.doc [Accessed 15 May 2013].

WHO (2009) *WHO guide to identifying the economic consequences of disease and injury*. Available from: http://apps.who.int/iris/handle/10665/137037 [Accessed 21 January 2016].

WHO (2013) *WHO methods and data sources for global burden of disease estimates 2000-2011* [online]. Available from: http://www.who.int/healthinfo/statistics/GlobalDALYmethods_2000_2011.pdf [Accessed 10 January 2016].

Wirodkul, T., Choldumrongkul, S., Kuprasert, S. and Wattanasit, S. (2008) Aflatoxin contamination in feedstuffs and complete feed for layer, broiler and swine in Songkhla province. *Mahidol University Journal*. 11(1), 8-18.

Wisselink, H.J., Smith, H.E., Stockhofe-Zurwieden, N., Peperkamp, K. and Vecht, U. (2000) Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. *Veterinary Microbiology*. 74(3), 237-248.

Wongkamma, A., Hinjoi, S. and Chumkasean, P. (2014) A situation of Streptococcus suis among humans in Thailand, 2011-2013. *Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health*. 45(21), 321-325.

Wongsawan, K., Gottschalk, M. and Tharavichitkul, P. (2015) Serotype- and virulence-associated gene profile of *Streptococcus suis* isolates from pig carcasses in Chiang Mai Province, Northern Thailand. *The Journal of Veterinary Medical Science*. 77(2), 233-236.

Wongsawan, K., Takenami, N., Pruksakorn, S., Fongcom, A., Gottschalk, M., Supjatura, V., Takai, S. and Tharavichitkul, P. (2006) Genetic diversity of *Streptococcus suis* isolated from pigs and humans in Chiang Mai and Lamphun province, Thailand. *International Congress Series*. 1289, 151-154.

World Bank (2016) *Health expenditure, total (% of GDP) | Data | Table*. Available from: http://data.worldbank.org/indicator/SH.XPD.TOTL.ZS [Accessed 22 January 2016].

World Bank (n.d.) *Rural population (% of total population) | Data | Table*. Available from: http://data.worldbank.org/indicator/SP.RUR.TOTL.ZS [Accessed 22 January 2016].

World Health Organisation (1983) *International health regulations (1969).* 3rd annotated ed. Geneva: Albany, N.Y: World Health Organization; Obtained from WHO Publications Centre USA.

World Health Organisation (ed.) (2008) *International health regulations: (2005)*. 2nd. ed. Geneva: WHO. pp. 74.

Wu, N.-H., Meng, F., Seitz, M., Valentin-Weigand, P. and Herrler, G. (2015) Sialic acid-dependent interactions between influenza viruses and *Streptococcus suis* affect the infection of porcine tracheal cells. *The Journal of General Virology*. 96(9), 2557-2568.

Xu, M., Wang, S., Li, L., Lei, L., Liu, Y., Shi, W., Wu, J., Li, L., Rong, F., Xu, M., Sun, G., Xiang, H. and Cai, X. (2010) Secondary infection with *Streptococcus suis* serotype 7 increases the virulence of highly pathogenic porcine reproductive and respiratory syndrome virus in pigs. *Virology Journal*. 7, 184. doi:10.1186/1743-422X-7-184

Ye, C., Bai, X., Zhang, J., Jing, H., Zheng, H., Du, H., Cui, Z., Zhang, S., Jin, D., Xu, Y., Xiong, Y., Zhao, A., Luo, X., Sun, Q., et al. (2008) Spread of *Streptococcus suis* sequence type 7, China. *Emerging Infectious Diseases*. 14(5), 787-791.

Ye, C., Zhu, X., Jing, H., Du, H., Segura, M., Zheng, H., Kan, B., Wang, L., Bai, X., Zhou, Y., Cui, Z., Zhang, S., Jin, D., Sun, N., et al. (2006) *Streptococcus suis* Sequence Type 7 Outbreak, Sichuan, China. *Emerging Infectious Diseases*. 12(8), 1203-1208.

Yiengprugsawan, V., Kelly, M., Seubsman, S. and Sleigh, A.C. (2010) The first 10 years of the Universal Coverage Scheme in Thailand: review of its impact on health inequalities and lessons learnt for middle-income countries. *Australasian Epidemiologist/Australasian Epidemiological Association*. 17(3), 24-26.

Yothayai, W., Wichit, A. and Chitwatchanan, K. (2006) Social context and consumption behaviour of people in village with botulism outbreak, Phayao province. *Lanna Public Health Journal*. 2(3), 216–229.

Youth encyclopedia (1994) pig raising in Thailand. In: *The Youth Encyclopedia* [online]. Bangkok: Available from: http://kanchanapisek.or.th/kp6/sub/book/book.php?book=18&chap=9&page=chap9.htm [Accessed 3 May 2016].

Yu, H., Jing, H., Chen, Z., Zheng, H., Zhu, X., Wang, H., Wang, S., Liu, L., Zu, R., Luo, L., Xiang, N., Liu, H., Liu, X., Shu, Y., et al. (2006) Human *Streptococcus suis* Outbreak, Sichuan, China. *Emerging Infectious Diseases*. 12(6), 914–920.

Yu, H., Liu, X., Wang, S., Liu, L., Zu, R., Zhong, W., Zhu, X., Xiang, N., Yuan, H., Meng, L., Ou, Y., Gao, Y., Lv, Q., Huang, Y., et al. (2005) [Matched case-control study for risk factors of human *Streptococcus suis* infection in Sichuan Province, China]. *Zhonghua Liu Xing Bing Xue Za Zhi, Zhonghua Liuxingbingxue Zazhi*. 26(9), 636639.

Zain, M.E. (2011) Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*. 15(2), 129–144.

Zhang, J., Zhu, J., Ren, H., Zhu, S., Zhao, P., Zhang, F., Lv, H., Hu, D., Hao, L., Geng, M., Gong, X., Pan, X., Wang, C. and Qi, Z. (2013) Rapid visual detection of highly pathogenic *Streptococcus suis* serotype 2 isolates by use of loop-mediated isothermal amplification. *Journal of Clinical Microbiology*. 51(10), 3250-3256.

Zhang, N. and He, Q.-S. (2015) Commensal Microbiome Promotes Resistance to Local and Systemic Infections. *Chinese Medical Journal*. 128(16), 2250-2255.

Zhang, P., Woodward, M., Shen, J. and Wu, Y. (2010) Individual Disability-Adjusted Life Year: A Summary Health Outcome Indicator Used for Prospective Studies. In: Victor R. Preedy and Ronald R. Watson (eds.). *Handbook of Disease Burdens and Quality of Life Measures* [online]. Springer New York. pp. 425–436. Available from: http://link.springer.com/referenceworkentry/10.1007/978-0-387-78665-0_24 [Accessed 6 February 2016].

Zhang, Y. and Wildemuth, B.M. (2009) Unstructured interviews. *Applications of Social Research Methods to Questions in Information and Library Science. Westport, CT: Libraries Unlimited* [online]. Available from: http://hsmi.psu.ac.th/upload/forum/Unstructured_interviews.pdf [Accessed 8 July 2014].

Zhu, W., Wu, C., Sun, X., Zhang, A., Zhu, J., Hua, Y., Chen, H. and Jin, M. (2013) Characterization of *Streptococcus suis* serotype 2 isolates from China. *Veterinary Microbiology*. 166 (3-4), pp. 527-534.

Appendix I - Economic burden: Interview result

Patient interview result

1. Patient characteristics (n=22)

Socio-demographic		**Income generation**	
Interviewee		***Main source of income***	
Patient	15	Agriculture	4
Not patient	7	Trader	8
Gender		Paid employment	3
Male	17	Daily hired labour	5
Female	5	Retirement pension	2
Age		***Household income (month)***	
0-15	0	0-9,250 baht (£0-185)	6
16-44	7	9251-11000 baht (£185-220)	5
45-64	12	11001-25000 baht (£220-500)	7
65 and over	3	More than 25000 baht (£500)	4
Marital status			
Single	5		
Married	13		
Divorced	1		
Widowed	3		
Household members			
1-2	9		
3-4	9		
5<	4		
Role in household			
Household head	14		
Spouse, son, daughter	8		
Level of Education			
None	1		
Primary level	10		
Secondary level	8		
Tertiary level	3		

2. Knowledge and Risk behavior

Knowledge of disease	
Moderate knowledge	17
Eat raw, eat raw or uncooked pork, eat raw traditional	
Good knowledge	2
Bacteria uncooked pork, blood	
Miss understanding	1
Immunodeficiency disease from food	
Don't know	2
How to prevent from disease	
Eat cooked	16
Eat pork from known source	1
No prevention	1
Don't know	4
Eat any raw dishes?	
No	1
Yes	21
Reason to eat raw dishes	
Delicious	13
Tradition, culture	4
Party, celebration	3
Easy to find	2
Alcohol consumption	
No	6
Everyday	5
3-5 per week	4
Once a week	2
Not often	5

3. Illness and treatment seeking behavior

First clinical signs	
Fever	17
Muscle pain	15
Diarrhea	10
Headache	18
Stiff neck	3
Seizure	3
Shock	4
Loss balance	12
Hearing loss	8
Deafness	1
Arthritis	2
Respiratory problem	4
Others (blurred vision, vomiting, shiver)	3
First treatment	
Self-medication	4
Private clinic	5
Health care center	1
Hospital	12
Duration from ill to visit hospital	
Less than one day	13
2-3 days	6
More than 3 days	3
Nearest hospital	
1-5 km	15
6-10 km	3
More than 10 km	4
Distance from home to treated hospital	
Less than 10 km	8
10-20 km	10
21-30 km	2
More than 30 km	2
Travel vehicle	
Motorbike	4
Own or relative car	14
Other people car	4
(eg. Neighbor, rescue, local administration)	

4. Cost before and during treatment paid by patients

Expense before visit hospital	
None	16
Up to 500 baht (£10)	4
2000 baht (£40)	1
Cost of travel to hospital	
Less than 100 baht (£2)	6
100-300 baht (£2-6)	13
More than 300 baht (£6)	1
Number of accompany person	
1	14
2	5
3	3
Cost of accompany person/day	
100-200 bath (£2-4)	9
201-300 baht (£4-6)	5
301-400 baht (£6-8)	2
401-500 baht (£8-10)	3
More than 500 baht (£10)	2
Total cost of accompany person	
Less than 1000 baht (£20)	5
1000-5000 baht (£20-100)	8
5001-10000 (£100-200)	4
More than 10000 baht (£200)	4
Admit duration	
Less than 10 days	9
10-30 days	11
More than 30 days	2
Health insurance	
National Health security	12
Social insurance	7
Government officer	3
Private insurance	1

Extra cost that patient have to pay	
None	17
Private room	1
Private hospital	3
Special examination	1
Special medicine and medical equipment (not include in insurance)	2
Borrow money	
No	16
Yes	6
Disability	
Full recovery	11
Dead	2
Hearing loss or deafness	6
Generalize weakness	2
Adhesive of joints	1
Work replacement	
Another adult in household	3
Hire labour (15-45 day	2
Nobody did work	14
Others (retired or too old to work)	2
Fallow up treatment	
No	7
Yes	13
N/A	2
Duration of follow up	
1-2 weeks	5
3-4 weeks	2
More than 4 weeks	6
Follow up cost	
None (paid by National health security)	12
10000 baht (£200)	1

recovery period	
1-2 weeks	2
3-4 weeks	8
2-6 months	1
More than 6 months (up to 1 year)	3
Not yet recovery	2
N/A	6

5. Impact from *S. suis* infection

Household Impact	
No impact	5
Psychological impact	9
Economic impact	8
Patient's impact	
Impact due to disability	5
Depress, worry	6
resignation	1
No impact	10

1. Hospital interview (n=6)

Size of hospital	
30 beds	3
60 beds	1
200 beds	1
800 beds	1
Staff	
General practitioner	
>5	3
5-10	2
<10	1
Specialist	
0	1
1-2	3
28	1
103	1
Nurse	
>50	3
50-100	1
100-200	1
>200 (453)	1
Assistance Nurse	
0	2
1	1
10	1
25	1
45	1
Epidemiologist	
1	1
2	3
3	2
Average patient per day	
100	1
300	3
>1000	2
***S. suis* laboratory**	
No	5
Yes	1

If do not have laboratory, how to diagnosis *S. suis*	
Send to other lab	4
Primary bacteria culture and clinical signs	1
Duration until diagnosis	
Primary diagnosis	1-2 days
confirmation diagnosis	1- 14 days
Refer patient to other hospital?	
No	2
Yes	4
Cost of refer patient	
Less than 500 baht (£10)	2
More than 1000 baht (£20)	2

Appendix II- Economic burden interview forms

No._____

The economic consequence estimation of Streptococcus suis in Chiang Mai, Thailand

Form 1 Patient Interview Guide

Interviewer instructions:

- Fill in numbers l__l__l – make sure you put a '0' if the answer is 'none' so we know the question has been answered
- Where there are choices, tick the box next to the answer given or fill in 'other' where relevant
- Write answer on lines_____

Interviewer Identification

1. Interview date (dd-mm-yy) l__l__l – l__l__l – l__l__l
2. Interviewer's name _____
3. Position _____

I. Socio-demographic characteristics

Section 1

1. Gender
    1. Male
    2. Female
2. Age l__l__l
3. Are you a *S. suis* patient?
    0. No
    1. Yes *(If 'yes', skip questions 5)*
4. What is your relationship with patient?
    1. Husband/wife
    2. Son/daughter
    3. Other_____

Section 2 Question about patient

5. Marital status
    1. Single
    2. Married
    3. Divorced
    4. Other_____

6. Religion
    1. Buddhism
    2. Christ
    3. Muslim
    4. Other_____
7. Education
    1. None
    2. Primary school
    3. Secondary school/Vocational certificate
    4. Diploma
    5. Further education
8. What is the patient position in the household?
    1. Household head
    2. Wife, husband, son, daughter, father, mother of household head
    3. Other family member
    4. Household employee
    5. Other_____

II. Description of household

Interviewer explains: We would like to know a bit about who lives in your household and types of work your household does.

9. How old is the household head? l__l__l
10. Is the household head
    1. Male
    2. Female
11. What is the highest level of education the household head completed, if interviewee not head of household?
    1. None
    2. Primary school
    3. Secondary school/Vocational certificate
    4. Diploma
    5. Further education
12. How many people live in your household? l__l__l
13. What are their age and sex?

Age (years)	Number of household members	
	Male	Female
0-4		
5-14		
15-64		
65+		

From which activities is the household's income?

14. What is the main occupation in your household, from which it gets most of its income?
    1. Agriculture (plant-crop farming)
    2. Agriculture (Animal farming)
    3. Trading (own business)
    4. Paid employment
    5. Daily paid employment
    6. Government officer
    7. Other_____
15. What is the second most important occupation?
    1. Agriculture (plant-crop farming)
    2. Agriculture (Animal farming)
    3. Trading (own business)
    4. Paid employment
    5. Daily paid employment
    6. Government officer
    7. Other_____
16. How many members of your household currently attend school? l__l__l
17. Can you please estimate the monthly and yearly income of your **household**?

Monthly	l__l__l__l__l__l__l
Yearly	l__l__l__l__l__l__l

III. Knowledge and health seeking behavior

Knowledge of *S. suis*

18. In your understanding, what is *S. suis*?

    _____

    _____
19. How people get infected? _____
20. How often do your family eat traditional dishes (Laab, Lu, Sa)_____
21. How do you prevent against *S. suis*? _____

    _____
22. Is there any control campaign conducted in your area at the moment (or in the past)?
    1. No
    2. Yes, how often? _____
23. Are there any people that you know who have had *S. suis*?
    0. No
    1. Yes

24. Which of the following dishes that you eat raw? (can choose more than 1)
    1. Laap (pork)
    2. Laap (cattle, buffalo)
    3. Lu
    4. Pla (pork)
    5. Pla (cattle, buffalo)
    6. Sa
    7. Nham
    8. Satay
    9. Steak
25. Why do you prefer to eat above raw dishes? _____
26. How often are you drinking alcohol?
    1. Everyday
    2. 3-5 days per week
    3. Once a week
    4. Not often
27. Have you or your family member had *S. suis* infection before?
    1. No
    2. Yes

Health seeking behavior during illness episode

28. When did you first begin experiencing symptoms for *S. suis*
    Date (DD/MM/YY) l__l__l/l__l__l/l__l__l
    Time Morning /Afternoon/Evening/Night
29. Where do you usually buy pork? _____
30. What symptoms did you first have? (can answer more than 1)
    1. Fever
    2. Muscle pain
    3. Acute diarrhea
    4. Ecchymosis
    5. Headache
    6. Stiff neck
    7. Seizer
    8. Shock
    9. Hyperventilation
    10. Balance loss
    11. Decrease hearing ability
    12. Hearing loss
    13. Arthritis
    14. Other, please specify_____
31. How long until you went to seek treatment? _____(days/weeks)
32. Where was the first place that you sought care?
    1. Self-medication (pharmacy/drug-seller/herbs)
    2. Home remedies
    3. Health center
    4. Private clinic
    5. Hospital (district/provincial/regional)
    6. Other, please specify_____

33. How long until you went to Healthcare center/hospital? _____
34. How far away is the nearest health center? l__l__l Km
35. How much did you pay before you go to health center/hospital?
    _____

IV. Impact of illness

Patient and patient's family costs – at the hospital/health center

36. How far from your house did you have to travel to reach the hospital/health center
    l__l__l Km
37. How did you get there?
    1. Walking
    2. Bicycle
    3. Car
    4. Bus
    5. Taxi (ex. Red taxi)
    6. Other, please specify _____
38. How much did it cost to go to hospital? _____
39. How long from your first symptoms to your diagnosis? l__l__l days
40. In what stage of healthcare center were you diagnosed?
    1. Primary health care center
    2. District hospital
    3. Provincial hospital
    4. Other, please specify _____
41. How long did you stay in the treatment center? l__l__l days
42. Who accompanied you at the hospital? _____
43. How much did it cost for the accompanied person _____
    (prompt: transportation, accommodation, food etc.)

44. What medicine were you given? _____
    _____
    _____
    (Prompt: traditional medicine, vitamins, antibiotics, painkillers, steroids, etc.)
45. Before discharge, did the hospital staff give you knowledge about *S. suis*?
    1. No
    2. Yes

46. What kind of insurance did you use to pay for the medication?
    1. Universal coverage scheme
    2. Social insurance
    3. Civil servant medical benefit scheme
    4. Private insurance
    5. No insurance
    6. Other, please specify _____
47. Were there any extra costs? (What else is paid for out of pocket)? Please specify and how much does it cost?_____
    _____
    _____
    (prompt: food, drug, travel, vigil over a sick cost etc.)
48. Did you have to borrow money to pay for these healthcare costs?
    1. No
    2. Yes
49. If yes, how much money did you have to borrow?_____

Household impact during treatment and recovery

50. While the patient is ill, who does the work he/she would normally do?
    1. Another adult in your household
    2. Someone from outside your household came and help
    3. Someone had to be paid to do it
    4. Nobody did your work
    5. Other, please specify _____
51. If someone was paid to do your work, how much were they paid and for how long?
    _____(amount)
    _____(days/weeks/months)
52. Do you face any disability after infection?
    1. No
    2. Yes, please specify_____
53. Did you have follow up treatment?
    1. No
    2. Yes (If 'Yes' answer number 54-56)
54. How long did you have to follow up treatment? _____
55. Did you have special treatment (such as cochlear implant)?
    1. No
    2. Yes
56. How much did the total follow up cost? _____

Impact of disability and death

57. How did your illness affect your household?

    _____
    _____
    _____

58. What did your family/community think about your illness?

    _____
    _____
    _____

59. Did your community/family/village help you in any way during or after your illness?

    _____
    _____
    _____
    _____

60. How long did it take for you to recover? _____(days/months)
61. If not yet recover, how are you felling now? _____
    _____
62. What was happening at home (to your household) while you were sick and recovering?

    _____
    _____

63. What have the long-term effects of illness been on the household?

    _____

Interview conclusion

We would like to thank you very much for your time and for this useful information.

64. Family members involve in the interview:

    _____
    _____
    _____
    _____

Summary

Before hospital cost	Hospital costs	Follow up cost	Loss of income costs	Total costs

ชุดที่ _____

การศึกษาผลกระทบทางเศรษฐกิจและสังคมจากการติดเชื้อเสตร็พโตคอกคัส ซูอิสของผู้ป่วยในจังหวัดเชียงใหม่

The economic and societal consequence estimation of Streptococcus suis in Chiang Mai, Thailand

แบบสัมภาษณ์ 1 : สำหรับการสัมภาษณ์ผู้ป่วย ญาติ หรือผู้ใกล้ชิด

คำแนะนำสำหรับผู้สัมภาษณ์:

- เติมตัวเลขในช่องว่าง l__l__l – ถ้าคำตอบเป็นศูนย์ ให้เติม '0' เพื่อให้ทราบว่าผู้สัมภาษณ์ได้ตอบคำถามแล้ว
- ถ้าคำถามเป็นตัวเลือก ให้วงกลมตัวเลข หรือเติมข้อความในช่องตามความเหมาะสม
- เขียนคำตอบในช่องว่างที่ให้ _____

ข้อมูลผู้สัมภาษณ์

1. วันที่สัมภาษณ์ (วัน-เดือน-ปี) l__l__l – l__l__l – l__l__l
2. ชื่อผู้สัมภาษณ์ _____
3. ตำแหน่ง _____

I. ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

ส่วนที่ 1

1. เพศ
    1. ชาย
    2. หญิง
2. อายุ l__l__l ปี
3. ผู้ตอบแบบสอบถามเป็นผู้ป่วยด้วยโรคเสตร็พโตคอกคัส ซูอิสใช่หรือไม่?
    2. ไม่ใช่
    3. ใช่ (ถ้า 'ใช่', ข้ามไปตอบข้อ 6)
4. ท่านมีความสัมพันธ์อย่างไรกับผู้ป่วย?
    1. สามี/ภรรยา
    2. บุตร
    3. อื่น ๆ _____

ส่วนที่ 2 คำถามเกี่ยวกับผู้ป่วย

5. สถานภาพการสมรส
    1. โสด
    2. สมรส
    3. หย่า
    4. อื่น ๆ _____
6. ศาสนา
    1. พุทธ
    2. คริสต์
    3. อิสลาม
    4. อื่น ๆ _____
7. การศึกษา
    0. ไม่ได้รับการศึกษา
    1. ระดับประถมศึกษา
    2. ระดับมัธยมศึกษา/ปวช./ปวส.
    3. การศึกษาระดับอนุปริญญา
    4. การศึกระดับปริญญาตรี หรือสูงกว่า
8. บทบาทของผู้ป่วยในครอบครัว?
    1. หัวหน้าครอบครัว
    2. ภรรยา, สามี, บุตร, บิดา/มารดา ของหัวหน้าครอบครัว
    3. ญาติ
    4. ลูกจ้าง
    5. อื่น ๆ _____

II. ข้อมูลเกี่ยวกับครอบครัว

ผู้สัมภาษณ์อธิบาย: เราต้องการทราบข้อมูลเล็กน้อยเกี่ยวกับบุคคลที่อาศัยอยู่ในครอบครัวของคุณ และอาชีพที่ทำ

9. อายุของหัวหน้าครอบครัว l__l__l ปี
10. หัวหน้าครอบครัวเป็น
    1. ชาย
    2. หญิง
11. ระดับการศึกษาสูงสุดของหัวหน้าครอบครัว?(กรณีที่ผู้ตอบแบบสอบถามไม่ใช่หัวหน้าครอบครัว)
    1. ไม่ได้รับการศึกษา
    2. ระดับประถมศึกษา
    3. ระดับมัธยมศึกษา/ปวช./ปวส.
    4. การศึกษาระดับอนุปริญญา
    5. การศึกระดับปริญญาตรี หรือสูงกว่า

12. ในครอบครัวมีผู้อาศัยกี่คน? l__l__l

13. จำนวนผู้อาศัยแบ่งตามอายุและเพศ?

อายุ (ปี)	จำนวนของสมาชิกในครอบครัว	
	ชาย	หญิง
0-4		
5-14		
15-64		
65+		

2. ที่มาของรายรับในครอบครัว

14. อาชีพที่เป็นรายได้หลักของครอบครัวคุณคือ?

1. การเกษตร (ทำสวน,ทำไร่)
2. เลี้ยงสัตว์
3. ธุรกิจ หรือค้าขาย (กิจการส่วนตัว)
4. พนักงานบริษัท/เอกชน (ได้รับเงินรายเดือน)
5. รับจ้างทั่วไป (ได้ค่าจ้างตามชั่วโมงทำงาน)
6. ข้าราชการ/รัฐวิสาหกิจ
7. อื่น ๆ _____

15. อาชีพรองลงมาคือ?

1. การเกษตร (ทำสวน,ทำไร่)
2. เลี้ยงสัตว์
3. ธุรกิจ หรือค้าขาย (กิจการส่วนตัว)
4. พนักงานบริษัท/เอกชน (ได้รับเงินรายเดือน)
5. รับจ้างทั่วไป (ได้ค่าจ้างตามชั่วโมงทำงาน)
6. ข้าราชการ/รัฐวิสาหกิจ
7. อื่น ๆ _____

16. มีสมาชิกในครอบครัวคุณกี่คนที่ยังศึกษาอยู่? l__l__l

17. รายได้ของครอบครัวในแต่ละเดือนและแต่ละปีประมาณเท่าไหร่?

รายเดือน l__l__l__l__l__l__l__l บาท

รายปี l__l__l__l__l__l__l__l บาท

III. ความรู้และพฤติกรรมของผู้ป่วยในการเข้ารับการรักษา

ความรู้ ทัศนคติ และการปฏิบัติเกี่ยวกับโรคเสตร็พโตคอกคัส ซูอิส

18. ในความเข้าใจของคุณโรคเสตร็พโตคอกคัส ซูอิส คือ?_____
19. คนเป็นโรคนี้ได้อย่างไร_____
20. ในครอบครัวของคุณมีการบริโภคลาบ หลู้ หรือไม่ บ่อยแค่ไหน?_____
21. คุณมีวิธีการป้องกันการติดเชื้อเสตร็พโตคอกคัส ซูอิส อย่างไร?

    _____
22. เคยมีการรณรงค์ป้องกันโรคเสตร็พโตคอกคัส ซูอิสในชุมชนของคุณหรือไม่?
    0. ไม่เคย
    1. เคย โปรดระบุความถี่ _____
23. คุณรู้จักใครที่เคยติดเชื้อเสตร็พโตคอกคัส ซูอิสหรือไม่?
    3. ไม่มี
    4. มี
24. คุณรับประทานอาหารชนิดใดต่อไปนี้ที่ไม่สุกบ้าง สามารถเลือกได้มากกว่า 1 ข้อ?
    1. ลาบหมู
    2. ลาบวัว-ควาย
    3. หลู้
    4. พล่าหมู
    5. พล่าวัว-ควาย
    6. ส้า
    7. แหนม
    8. สะเต๊ะ
    9. สเต็ค
25. ทำไมคุณถึงนิยมบริโภคอาหารตามข้อ 27 _____
26. คุณดื่มแอลกอฮอล์บ่อยแค่ไหน?
    1. ทุกวัน
    2. อาทิตย์ละ 3-5 วัน
    3. อาทิตย์ละครั้ง
    4. นาน ๆ ครั้ง
27. คุณหรือคนในครอบครัวเคยติดเชื้อเสตร็พโตคอกคัส ซูอิส มาก่อนหรือไม่?
    0. ไม่เคย
    1. เคย

การเข้ารับการรักษาของผู้ป่วย

28. คุณเริ่มมีอาการป่วยเมื่อไหร่

    วันที่ (วัน/เดือน/ปี) l__l__l/l__l__l/l__l__l

    เวลา เช้า / กลางวัน / เย็น / กลางคืน

29. โดยปกติคุณซื้อเนื้อสุกรจากที่ไหน? _____

30. เริ่มแรกคุณมีอาการป่วยอย่างไร (สามารถตอบได้มากกว่า 1 ข้อ)

    1. ไข้
    2. ปวดกล้ามเนื้อ
    3. ท้องเสีย
    4. มีจ้ำเลือด
    5. ปวดศีรษะ
    6. คอแข็ง
    7. ชัก
    8. ช็อค
    9. เสียการทรงตัว
    10. ได้ยินลดลง
    11. หูหนวก
    12. ข้ออักเสบ
    13. หายใจหอบ
    14. อื่น ๆ โปรดระบุ_____

31. นานเท่าไหร่กว่าคุณจะเริ่มรับการรักษา? _____(วัน/สัปดาห์)

32. คุณเริ่มการรักษาจากที่ไหน?

    1. หายารับประทานเอง (ร้านขายยา/ยาสมุนไพร)
    2. หมอพื้นบ้าน
    3. คลินิกเอกชน
    4. โรงพยาบาลส่งเสริมสุขภาพ
    5. โรงพยาบาล (อำเภอ/จังหวัด/เอกชน)
    6. อื่น ๆ โปรดระบุ _____

33. คุณรอนานเท่าไหร่จึงจะเข้ารับการรักษาจากบุคลากรทางการแพทย์?

    _____

34. ระยะทางจากบ้านคุณและสถานบริการสุขภาพที่ใกล้ที่สุด? l__l__l กิโลเมตร

35. คุณมีค่าใช้จ่ายก่อนก่อนที่จะเข้ารับการรักษาจากสถานพยาบาลเป็นจำนวนเท่าไหร่?

    _____

IV. ผลกระทบจากการป่วย

ค่าใช้จ่ายของผู้ป่วยและครอบครัว – ที่สถานพยาบาล

36. ระยะทางระหว่างบ้านกับสถานพยาบาลที่คุณเข้ารับการรักษาห่างกัน l__l__l กิโลเมตร

37. คุณเดินทางไปสถานพยาบาลอย่างไร?

    1. เดิน
    2. จักรยาน
    3. รถยนต์
    4. รถโดยสารประจำทาง
    5. รถรับจ้าง (เช่น รถแดง)
    6. อื่น ๆ โปรดระบุ _____

38. ค่าใช้จ่ายสำหรับเดินทางไปสถานพยาบาลเป็นเงินเท่าไหร่ ? _____

39. คุณได้รับการวินิจฉัยว่าเป็นโรคเสตร็พโตคอกคัส ซูอิส หลังจากมีอาการนานแค่ไหน? l__l__l วัน

40. คุณได้รับการวินิจฉัยว่าเป็นโรคเสตร็พโตคอกคัส ซูอิส ที่ไหน?

1. โรงพยาบาลส่งเสริมสุขภาพตำบล
2. โรงพยาบาลระดับอำเภอ
3. โรงพยาบาลระดับจังหวัด
4. อื่น ๆ โปรดระบุ _____

41. คุณต้องนอนที่สถานพยาบาลนานกี่วัน ? l__l__l วัน

42. ระหว่างที่คุณนอนโรงพยาบาลมีคนดูแลกี่คน? _____

43. ค่าใช้จ่ายของผู้มาดูแลเป็นจำนวนเท่าไหร่_____

(ยกตัวอย่าง : ค่าเดินทาง ค่าที่พัก ค่าอาหารต่อวัน เป็นต้น)

44. คุณได้รับยาอะไรบ้างระหว่างการรักษา? _____

_____

_____

(*ตัวอย่าง : ยาปฏิชีวนะ ยาแก้ปวด สเตียรอยด์ วิตามิน สมุนไพร ฯลฯ*)

45. ก่อนออกจากสถานพยาบาลมีเจ้าหน้าที่ให้ความรู้คุณเกี่ยวกับโรคเสตร็พโตคอกคัส ซูอิสหรือไม่?

0. ไม่มี
1. มี

46. คุณมีสิทธิในการรักษาพยาบาลแบบใด?

1. บัตรประกันสุขภาพ (บัตรทอง)
2. ประกันสังคม
3. สิทธิข้าราชการ/รัฐวิสาหกิจ
4. ประกันสุขภาพเอกชน
5. ชำระเองไม่มีสิทธิใด ๆ
6. อื่น ๆ โปรดระบุ _____

47. ถ้าคุณมีสิทธิในการรักษาพยาบาล คุณมีค่าใช้จ่ายอื่น ๆ ที่ต้องจ่ายเพิ่มหรือไม่? (มีค่าใช้จ่ายอะไรบ้างที่คุณต้องจ่ายเอง)

โปรดระบุค่าใช้จ่ายและจำนวนเงินที่จ่าย

_____

_____

_____

(*ตัวอย่าง : ค่าอาหาร ค่ายา ค่าห้อง ค่าอุปกรณ์ทางการแพทย์อื่น ๆ ฯลฯ*)

48. คุณต้องยืมเงินมาเพื่อจ่ายค่าใช้จ่ายส่วนนี้หรือไม่?

0. ไม่
1. ใช่

49. ถ้าใช่ คุณยืมเงินมาเป็นจำนวนเท่าไหร่?_____

ผลกระทบต่อครอบครัวระหว่างการรักษาและพักฟื้น

50. ระหว่างที่ผู้ป่วยรับการรักษามีใครทำงานแทน?
    1. บุคคลอื่นในครอบครัว
    2. ญาติ
    3. จ้างผู้อื่นทำงานแทน
    4. ไม่มีใครทำงานแทน
    5. อื่น ๆ โปรดระบุ _____
51. ถ้าต้องจ้างผู้อื่นทำงานแทน จะต้องจ่ายค่าจ้างเท่าไหร่ เป็นระยะเวลาเท่าไหร่?
    _____(บาท)
    _____(วัน / เดือน / ปี)
52. คุณมีความผิดปกติทางร่างกายหลังจากการติดเชื้อหรือไม่ ?
    0. ไม่
    1. ใช่ โปรดระบุ _____
53. คุณต้องรับการรักษาต่อเนื่องหลังจากออกจากสถานพยาบาลหรือไม่?
    0. ไม่ (*ข้ามไปข้อ 53*)
    1. ใช่
54. คุณต้องรับการรักษาต่อเนื่องนานเท่าไหร่?_____
55. คุณต้องเข้ารับการรักษาเป็นพิเศษหรือไม่ (เช่น การฝังประสาทหูเทียม)?
    0. ไม่
    1. ใช่
56. คุณต้องจ่ายค่าใช้จ่ายในการรักษาต่อเนื่องเท่าไหร่? _____

ผลกระทบเนื่องจากความผิดปกติหลังจากการติดเชื้อ หรือเสียชีวิต

57. การป่วยส่งผลกระทบต่อครอบครัวของคุณอย่างไร?
    _____
    _____
    _____
58. ครอบครัวหรือสังคมรอบตัวคุณคิดอย่างไรกับการป่วยของคุณ?
    _____
    _____
    _____
59. ครอบครัว สังคม และชุมชนของคุณช่วยเหลือคุณอย่างไรหลังจากคุณป่วย?
    _____
    _____
    _____

60. ใช้ระยะเวลานานเท่าไหร่กว่าคุณจะหายเป็นปกติ? _____(วัน/ เดือน)
61. ถ้ายังไม่หายเป็นปกติ ตอนนี้คุณรู้สึกอย่างไรบ้าง? _____

_____

62. ครอบครัวคุณเป็นอย่างไรบ้างระหว่างที่คุณป่วยและพักฟื้น?

_____

_____

63. มีผลกระทบระยะยาวเนื่องจากการป่วยของคุณต่อครอบครัวหรือไม่?

_____

สรุปการสัมภาษณ์

ขอขอบคุณเป็นอย่างมากที่สละเวลาในการตอบคำถามและให้ข้อมูลที่เป็นประโยชน์

64. สมาชิกครอบครัวที่ร่วมในการสัมภาษณ์:

_____

_____

_____

_____

Summary

Before hospital cost	Hospital costs	Follow up cost	Loss of income costs	Total costs

No._____

The economic consequence estimation of *Streptococcus suis* in Chiang Mai, Thailand

Form 2 Health center Interview Guide

Interviewer instructions:

- Fill in numbers l__l__l – make sure you put a '0' if the answer is 'none' so we know the question has been answered
- Where there are choices, tick the box next to the answer given or fill in 'other' where relevant
- Write answer on lines_____

- **Interviewer Identification**

1. Interview date (dd-mm-yy) l__l__l – l__l__l – l__l__l
2. Interviewer's name _____
3. Position _____

I. Socio-demographic characteristics

Section 1 -Interviewee

1. Please describe your duty

   _____
   _____
   _____
   _____

2. How long do you work here? l__l__l years

Section 2 -Treatment center

3. Name of hospital _____
4. How many beds do the hospital has?
   1. 30 beds
   2. 60 beds
   3. 120 beds
   4. 600 beds
5. How much population do the services cover?

   Population _____heads
   _____households

   Area _____

6. Number of personnel in this treatment center? please specify

General practitioner	l__l__l__l	heads
Specialist	l__l__l__l	heads
Nurse practitioner	l__l__l__l	heads
Nurse assistant	l__l__l__l	heads
Public health/epidemiologist	l__l__l__l	heads

7. How many average patients per day? l__l__l__l__l heads

II. *S. suis* patient's costs at healthcare center

8. How many *S. suis* cases presented for treatment in the past 2 years? l__l__l
9. How many *S. suis* patients admitted at this hospital? l__l__l
10. Do you have laboratory diagnosis for *S. suis*?
    1. No
    2. Yes *(If 'Yes' skip number 13)*
11. If you don't have laboratory how do you diagnose *S. suis*?

    _____

    _____
12. How long from patient presented until *S. suis* was diagnosed?

    _____
13. Please describe treatment/medicine you had given to patients?

    _____

    _____

    _____

    _____

    _____
14. Did you refer patients to more facilities hospital?
    1. No
    2. Yes
15. What was the criterion to refer patient? _____
16. How pay for transfer cost? _____
17. Could you estimate the transfer cost? _____
18. Could you estimate healthcare costs for in/out *S. suis* patient?

    _____

    _____
19. Could you estimate follow up costs for patient?

    _____

    _____

    (prompt: examination cost, medicine, laboratory, etc.)
20. Were there any extra costs that happened from patient?

    _____

    _____

21. Could you estimate the cost of disease investigation and control? Please specify number of manpower, day and other expenses.

_____

_____

_____

(prompt: transportation cost, daily wage, knowledge transfer: brochure, poster, etc)

22. How much did you average charge to *S. suis* patients?

_____

23. What were payment method of the patients?
    1. Universal health security scheme
    2. Social insurance
    3. Civil servant medical benefit scheme
    4. Private insurance
    5. Cash (no insurance)
    6. Other, please specify_____

Summary

Treatment cost	Extra cost	Total cost	Patient's payment

ชุดที่_____

การศึกษาผลกระทบทางเศรษฐกิจและสังคมจากการติดเชื้อเสตร็พโตคอกคัส ซูอิสของผู้ป่วยในจังหวัดเชียงใหม่

The economic and societal consequence estimation of *Streptococcus suis* in Chiang Mai, Thailand

แบบสัมภาษณ์ 2 : สำหรับการสัมภาษณ์บุคลากรทางการแพทย์

คำแนะนำสำหรับผู้สัมภาษณ์ :

- เติมตัวเลขในช่องว่าง l__l__l – ถ้าคำตอบเป็นศูนย์ ให้เติม '0' เพื่อให้ทราบว่าผู้สัมภาษณ์ได้ตอบคำถามแล้ว
- ถ้าคำถามเป็นตัวเลือก ให้วงกลมตัวเลข หรือเติมข้อความในช่องตามความเหมาะสม
- เขียนคำตอบในช่องว่างที่ให้ _____

ข้อมูลผู้สัมภาษณ์

1. วันที่สัมภาษณ์ (วัน-เดือน-ปี) l__l__l – l__l__l – l__l__l
2. ชื่อผู้สัมภาษณ์ _____
3. ตำแหน่ง _____

I. ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

ส่วนที่ 1

1. กรุณาระบุลักษณะงานของท่าน

   _____
   _____
   _____
   _____

2. ท่านทำงานที่นี่มานานเท่าไหร่? l__l__l ปี

ส่วนที่ 2 –ข้อมูลสถานบริการสุขภาพ

3. ชื่อของสถานพยาบาล _____
4. โรงพยาบาลท่านเป็นโรงพยาบาลกี่เตียง?
   1. 30 เตียง
   2. 60 เตียง
   3. 120 เตียง
   4. 600 เตียง

5. ความครอบคลุมการให้บริการ?

ประชากร _____ราย

_____ครัวเรือน

พื้นที่ _____

6. จำนวนบุคลากรทางการแพทย์ในสถานพยาบาลแห่งนี้ โปรดระบุ

แพทย์ทั่วไป	l__l__l__l	คน
แพทย์เฉพาะทาง	l__l__l__l	คน
พยาบาลวิชาชีพ	l__l__l__l	คน
ผู้ช่วยพยาบาล	l__l__l__l	คน
เจ้าหน้าที่สาธารณสุข/ระบาดวิทยา	l__l__l__l	คน

7. จำนวนเฉลี่ยของผู้ป่วยที่เข้ารับบริการแต่ละวัน? l__l__l__l__l คน

II. ค่าใช้จ่ายของผู้ป่วยโรคเสตร็พโตคอกคัส ซูอิสที่สถานบริการสุขภาพ

8. ในช่วง 2 ปีที่ผ่านมามีผู้ป่วยโรคเสตร็พโตคอกคัส ซูอิสเข้ารับบริการกี่ราย? l__l__l ราย

9. ผู้ป่วยโรคเสตร็พโตคอกคัส ซูอิสที่ต้องพักรักษาตัวกี่ราย? l__l__l ราย

10. ที่สถานพยาบาลแห่งนี้มีห้องปฏิบัติการเพื่อวินิจฉัยโรคเสตร็พโตคอกคัส ซูอิสหรือไม่?

1. ไม่มี
2. มี *(ข้ามไปข้อ 14)*

11. ถ้าไม่มีห้องปฏิบัติการ คุณทำการวินิจฉัยโรคเสตร็พโตคอกคัส ซูอิส อย่างไร?

_____

_____

12. ต้องใช้ระยะเวลานานเท่าไหร่ตั้งแต่ผู้ป่วยเข้ารับการรักษาในการวินิจฉัยโรค?

_____

13. โปรดระบุวิธีการรักษาและยาที่ให้แก่ผู้ป่วย?

_____

_____

_____

_____

_____

14. มีการส่งต่อผู้ป่วยไปยังโรงพยาบาลที่มีความพร้อมมากกว่าหรือไม่?

1. ไม่
2. ใช่

15. ใช้แนวทางปฏิบัติใดเป็นเกณฑ์การตัดสินใจในการส่งต่อผู้ป่วย?_____

_____

16. ใครเป็นผู้ออกค่าใช้จ่ายในการส่งต่อผู้ป่วย_____

17. ค่าใช้จ่ายโดยประมาณในการส่งต่อผู้ป่วย_____

18. ค่าใช้จ่ายโดยประมาณของสถานพยาบาลในการรักษาผู้ป่วยใน/นอกด้วยโรคเสตร็พโตคอกคัส ซูอิส?

_____

_____

19. ค่าใช้จ่ายโดยประมาณในการรักษาต่อเนื่องของผู้ป่วยโรคเสตร็พโตคอกคัส ซูอิส?

_____

_____

(ยกตัวอย่าง : ค่าตรวจ ค่ายา ค่าแลป ค่าใช้จ่ายอื่น ๆ เป็นต้น)

20. มีค่าใช้จ่ายอื่น ๆ นอกเหนือจากที่กล่าวมาแล้วหรือไม่? เช่น อุปกรณ์เสริม หูฟัง กรณีหูดับ

_____

_____

21. ค่าใช้จ่ายในการจัดการควบคุมและป้องกันโรค โปรดระบุจำนวนคน จำนวนวัน และค่าใช้จ่ายอื่นที่เกิดขึ้น

_____

_____

_____

(ยกตัวอย่าง : ค่าเดินทางไปสอบสวนและควบคุมโรค การค้นหาผู้ป่วยเพิ่มเติมในชุมชน ค่าติดต่อประสานงานกับผู้นำชุมชน/ท้องถิ่น การให้ความรู้ประชาชน การออกรณรงค์ให้ความรู้ เป็นต้น)

22. ค่ารักษาที่คนไข้ต้องจ่ายให้โรงพยาบาลโดยเฉลี่ยเท่าไหร่?

_____

23. ผู้ป่วยมักมีสิทธิการรักษาพยาบาลแบบใด

1. บัตรประกันสุขภาพ (บัตรทอง)
2. ประกันสังคม
3. สิทธิข้าราชการ/รัฐวิสาหกิจ
4. ประกันสุขภาพเอกชน
5. ชำระเองไม่มีสิทธิใด ๆ
6. อื่น ๆ โปรดระบุ _____

สรุปการสัมภาษณ์

ขอขอบคุณเป็นอย่างมากที่สละเวลาในการตอบคำถามและให้ข้อมูลที่เป็นประโยชน์

Summary

Treatment cost	Extra cost	Total cost	Patient's payment